DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
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# *HELICOBACTER PYLORI* INFECTION IN CHILDREN: EPIDEMIOLOGICAL AND THERAPEUTIC ASPECTS

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*To my family*

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# 1. LIST OF ORIGINAL PUBLICATIONS

I Vorobjova T, Grünberg H, Oona M, Maaroos HI, Nilsson I, Wadström T, Covacci A, Uibo R. Seropositivity to *Helicobacter pylori* and CagA protein in schoolchildren of different ages living in urban and rural areas in southern Estonia. Eur J Gastroenterol Hepatol. 2000;12:97–101.

II Oona M, Utt M, Nilsson I, Uibo O, Vorobjova T, Maaroos HI. *Helicobacter pylori* infection in children in Estonia: decreasing seroprevalence during the 11-year period of profound socioeconomic changes. Helicobacter. 2004;9:233–41.

III Oona M, Rägo T, Maaroos HI. Long-term recurrence rate after treatment of *Helicobacter pylori* infection in children and adolescents in Estonia. Scandinavian Journal of Gastroenterology. 2004;39:1186–91.

IV Oona M, Rägo T, Maaroos HI, Mikelsaar M, Lõivukene K, Salminen S, Korhonen H. *Helicobacter pylori* in children with abdominal complaints: has immune bovine colostrum some influence on gastritis? Alpe Adria Microbiology Journal. 1997;6:49–57.

V Oona M. *Helicobacter pylori* infection and birth cohort phenomenon. Eesti Arst. 2004;83:458–62 (In Estonian).

# 2. ABBREVIATIONS

| | |
|---|---|
| BIC | bovine immune colostrum |
| *CagA* | cytotoxin-associated gene |
| CagA | cytotoxin-associated protein |
| cfu | colony forming unit |
| DNA | desoxyribonucleic acid |
| ELISA | enzyme-linked immunosorbent assay |
| ESPCG | European Society for Primary Care Gastroenterology |
| *H. pylori* | *Helicobacter pylori* |
| IARC | International Agency for Research on Cancer |
| ICD | International Classification of Diseases |
| kb | kilobase |
| kDa | kilodalton |
| MALT | mucosa associated lymphoid tissue |
| $M_r$ | relative molecular mass |
| NASPGN | North American Society for Pediatric Gastroenterology and Nutrition |
| NCTC | national collection of type cultures |
| NA | not addressed |
| NS | non-significant |
| OR | odds ratio |
| PAI | pathogenicity island |
| PCR | polymerase chain reaction |
| RAA | relative antibody activity |
| RAP | recurrent abdominal pain |
| SD | standard deviation |
| UBT | urea breath test |
| VacA | vacuolating cytotoxin |
| *vacA* | vacuolating cytotoxin gene |

# 3. INTRODUCTION

Identification of *Helicobacter pylori* as an infectious agent, responsible for chronic gastritis and peptic ulcer disease, has completely changed the understanding of etiopathogenesis and management of upper gastrointestinal diseases.

The prevalence of *H. pylori* infection varies, being almost universal in many developing countries and less prevalent in industrialized countries; yet at least one third of the populations of United States, Western Europe and Oceania are infected (Parsonnet 1998). In Estonia, the seroprevalence of *H. pylori* infection among adults is high, >80% (Vorobjova *et al.* 1994, Lindkvist *et al.* 1999a). Also, the incidence of peptic ulcer, especially among young patients, and incidence of gastric cancer is high in Estonia, compared with that observed in Western Europe (Kolk *et al.* 2002, Estonian Cancer Registry & Estonian Cancer Centre 2003).

Although the gastroduodenal diseases associated with *H. pylori* infection are principally manifested in adulthood, the process that leads to morbidity starts in childhood, as childhood is the critical period for acquisition of the infection. To understand epidemiology, pathogenesis, natural course and clinical manifestations of *H. pylori* infection and to find optimal management of the infection, studies on pediatric populations are necessary.

In Estonia, there are long tradition, ample experience and high competence in the research of chronic gastritis as well as *H. pylori* infection. Community-based epidemiological studies of chronic gastritis were initiated by Prof. Kaljo Villako more than 30 years ago (Kekki *et al.* 1977, Villako *et al.* 1982). Studies of *H. pylori* infection in Estonia started soon after the publication of the results of successful isolation of a new bacterium in the gastric mucosa, known now as *H. pylori (*Marshall & Warren 1984). The first doctoral dissertation in medicine, defended at the University of Tartu since 1991, after the regaining of Estonia's independence, was a study of the natural course of gastric ulcer in connection with chronic gastritis and *H. pylori* (Maaroos 1991a). Since that time, altogether 11 doctoral theses addressing different aspects of *H. pylori* infection have been defended at the University of Tartu.

My interest in *H. pylori* infection arose from my interest in pediatric infectious diseases and from my work experience at the Department of Infectious Diseases of the Children's Clinic of Tartu University Clinics. The aim of the present study was to explore the epidemiological aspects of *H. pylori* infection among children in Estonia: the prevalence of the infection and the changes in the prevalence during a period of profound socioeconomic changes; as well as the therapeutic aspects of *H. pylori* infection in children: long-term results of antibacterial treatment and the effectiveness of another therapeutic approach, administration of specific bovine immune colostrum.

# 4. REVIEW OF THE LITERATURE

## 4.1. Epidemiology of *H. pylori* infection

### 4.1.1. Prevalence of *H. pylori* infection

*H. pylori* infection is one of the commonest infections worldwide, occurring in all regions and infecting at least half of the world's population (Parsonnet 1998). However, the prevalence of *H. pylori* infection varies widely among and within populations of different countries.

Comparison of the prevalence rates in different populations requires consideration of some methodological issues. As *H. pylori* prevalence has declined over the time in many countries (see Chapter 4.1.3.), the time when the prevalence was determined has to be taken into account. The diagnostic accuracy of different methods of detecting *H. pylori* infection may vary among different age groups, *e.g.* use of serological tests may lead to underestimation of the true prevalence in young children due to the lower sensitivity of the test in this age group (Raymond *et al.* 1996, Khanna *et al.* 1998, Kindermann *et al.* 2001). Only a few studies have focused on a random sample of subjects selected from the general population of the whole country: such studies have been conducted, *e.g.* in the USA, Mexico and Germany (Staat *et al.* 1996, Torres *et al.* 1998, Seher *et al.* 2000). Local studies may not necessarily be applicable to the whole population of a country, as there may exist regional (Stroffolini *et al.* 1998, Broutet *et al.* 2001), urban-rural (Dore *et al.* 2002), or ethnic (Rothenbacher *et al.* 1998, Boey *et al.* 1999) differences in the prevalence of *H. pylori* infection. Studies that have involved blood donors or attendees of health check-up visits may not be representative of general population in terms of the socioeconomic status.

Still, comparison of the prevalence rates of *H. pylori* infection throughout the world clearly reveals two common features. The first common feature is that the infection is more prevalent in poor socioeconomic conditions: the prevalence is higher in developing countries than in industrialized countries (Mégraud *et al.* 1989), while within countries, the prevalence is higher among groups with lower a socioeconomic status (Graham *et al.* 1991, The EUROGAST Study Group 1993, Murray *et al.* 1997, Reshetnikov *et al.* 2003). The second common feature is that the prevalence increases with age (Mégraud *et al*. 1989, The EUROGAST Study Group 1993) whereas two different patterns are discernible (Pounder & Ng 1995): the first pattern is generally noted in developing countries and is characterized by rapid increase in the prevalence during the first decades of life and an almost uniformly high prevalence throughout adulthood (Figure 1). The second pattern is mainly observed in industrialized

countries and is characterized by low prevalence among children and steady gradual increase in the prevalence with advanced age (Figure 2).

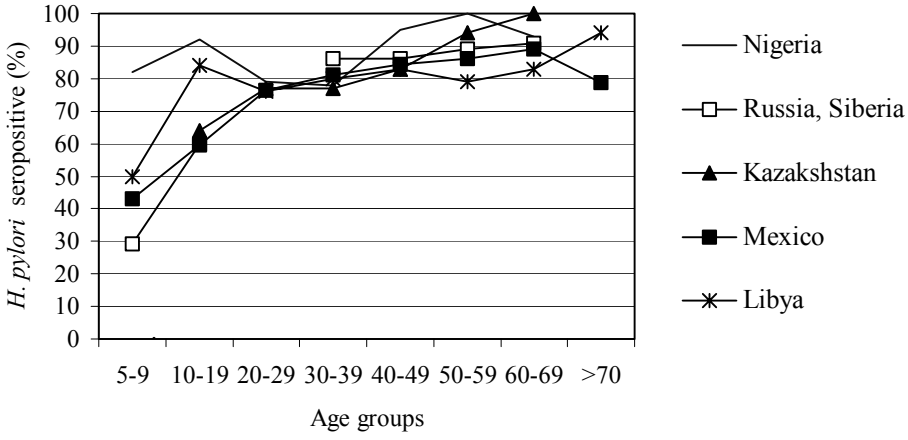


**Figure 1.** Prevalence of *H. pylori* infection: rapid increase in the prevalence during the first decades of life and an almost uniformly high prevalence throughout adulthood (pattern I). The figure is based on the data from the following papers: Holcombe *et al.* 1992, Reshetnikov *et al.* 2001, Nurgalieva *et al.* 2002, Torres *et al.* 1998, Bakka & Salih 2002.

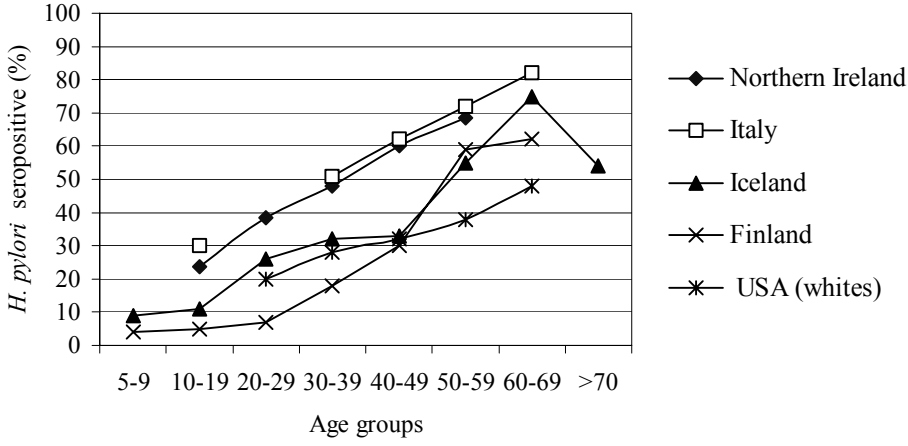


**Figure 2.** Prevalence of *H. pylori* infection: low prevalence among children and steady gradual increase in the prevalence with advanced age (pattern II). The figure is based on data from the following papers: Murray *et al.* 1997, Dominici *et al.* 1999, Bergenzaun *et al.* 1996, Rehnberg-Laiho *et al.* 1998, Kosunen *et al.* 1997, Graham *et al.* 1991.

At the present time, the prevalence of *H. pylori* infection in many Western European countries is considerably lower than 50% for adults and lower than 20% for children; much higher prevalences occur in countries of Africa, Asia, and South America (Figure 3).

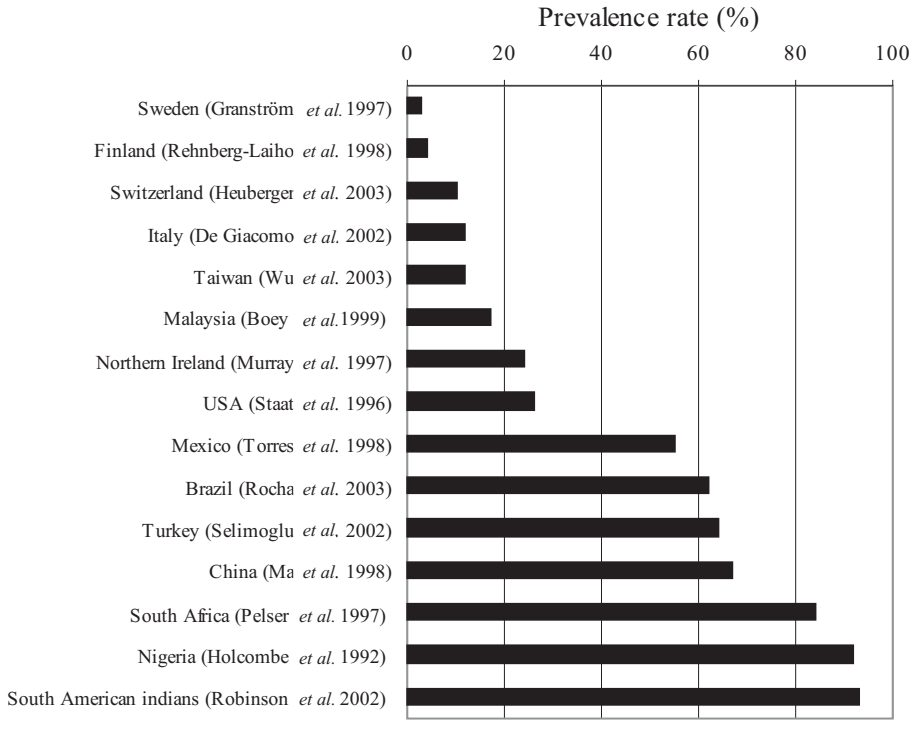


**Figure 3.** Prevalence of *H. pylori* infection in different countries among children between 10–16 years of age

The prevalence rates can be strikingly different within one and the same geographical region; for example, in the Baltic Sea region, the prevalence rates are higher in the countries lying on the eastern coast (Estonia, Russia, Latvia, Poland) compared with the other countries in that region (Finland, Sweden, Denmark, Germany) (Table 1). *H. pylori*-positivity in adults is more closely associated with living conditions and with the parents' socioeconomic status in childhood than with current living conditions and socioeconomic status (Mendall et al.1992, Malaty & Graham 1994, Malaty *et al.* 1998), suggesting that childhood is a period of major risk for acquisition of *H. pylori* infection. In Estonia, the prevalence of *H. pylori* infection among children, aged 4–15 years, with abdominal complaints was 58% (Maaroos *et al.* 1991b), while the prevalence of *H. pylori* infection in general children population was not known and was planned to be determined in the present study.

**Table 1.** *H. pylori* prevalence rates for children and adults in the countries of the Baltic Sea region

| Country | Reference | Study population | Age (years) | Prevalence rate (%) | |
|---|---|---|---|---|---|
| Estonia | Vorobjova *et al.* 1994 | inhabitants of a village | 15–19<br>20–29<br>30–39<br>40–49<br>50–59 | 69<br>83<br>89<br>90<br>91 | |
| | Lindkvist *et al.* 1999a | mothers of infants living in island Saaremaa | mean age 24, range 17–43 | 83 | |
| Russia | Malaty *et al.* 1996a | various non-random populations living in St. Petersburg region | 1–9<br>10–19<br>20–29<br>30–39<br>40–49 | 36<br>50<br>88<br>84<br>91 | |
| Latvia | Daugule *et al.* 2001 | visitors of healthcare centers in Riga | 1–8<br>9–12 | 16<br>32 | |
| Poland | Czkwianianc *et al.* 1996 | randomly selected urban children | 3–5<br>6–10<br>11–17 | 17<br>28<br>42 | |
| | Matysiak-Budnik *et al.* 1996 | outpatients, blood donors, students | <5<br>20–25<br>26–80 | 18<br>80<br>80–100 | |
| Finland | Ashorn *et al.* 1996 | randomly selected children | 2 | 5 | |
| | Rehnberg-Laiho *et al.* 1998 | vaccine trial participants | 14–15 | 4 | |
| | Kosunen *et al.* 1997 | randomly selected inhabitants of a semiurban community | 15–24<br>25–34<br>35–44<br>45–54 | 5<br>7<br>18<br>30 | |
| Sweden | Tindberg *et al.* 2001a | schoolchildren from Stockholm | 10–12 | 2 (Scandinavian parents) | |
| | Bergenzaun *et al.* 1996 | persons attending primary health care centers, Southern Sweden | 10–19<br>20–29<br>30–39<br>40–49 | 7<br>7<br>17<br>31 | |
| Denmark | Wewer *et al.* 1998 | outpatients | 3–15 | 10 | |
| Germany | Rosenstock *et al.* 2000 | randomly selected persons from Copenhagen county | 40<br>50<br>60 | 12<br>26<br>27 | |
| | Rothenbacher *et al.* 1998 | preschool children of Ulm region | 5–7 | 6 (German)<br>45 (Turkish) | |
| | Seher *et al.* 2000 | randomly selected persons | 18–29<br>30–39<br>40–49 | West Germany<br>21<br>28<br>37 | East Germany<br>25<br>39<br>54 |

### 4.1.2. Incidence of *H. pylori* infection

As acute *H. pylori* infection has no specific signs and symptoms, it is normally not detected at onset. In adults, the incidence rates are mostly derived from retrospective longitudinal serosurveys, and these studies are mostly conducted in industrialized countries. It appears that acquisition of the infection during adulthood is a rare event: seroconversion (*i.e.* change of the serostatus from seronegative to seropositive) occurs generally at a rate less than 1% per year of follow-up. Seroreversion (*i.e.* change of the serostatus from positive to negative) appears to occur approximately at the same or even higher rate than seroconversion (Table 2).

**Table 2.** Seroconversion and seroreversion rates of *H. pylori* infection for adults

| Reference | Country | Duration of follow-up (years) | Seroconversion rate, % per year of follow-up | Seroreversion rate, % per year of follow-up |
|---|---|---|---|---|
| Parsonnet *et al.* 1992 | USA | mean 8.5 | 0.5 | |
| Kuipers *et al.* 1993 | Netherlands | mean 11.5 | 0.3 | |
| Cullen *et al.* 1993 | Australia | 21 | 0.3 | 1.0* |
| Sipponen *et al.* 1996 | Finland | 15 | 0.4 | |
| Kosunen *et al.* 1997 | Finland | 21 | 0.2* | 0.2* |
| Kumagai *et al.* 1998 | Japan | mean 7 | 1.0 | 1.5 |
| Rosenstock *et al.* 2000 | Denmark | 11 | 0.1* | 0.7* |
| Menegatti *et al.* 2000 | Italy | 5 | 0.3* | |
| Kikuchi *et al.* 2004 | Japan | 9 | 0.7 | 0.8 |

*calculated according to the data presented in the paper

Data about the incidence of *H. pylori* infection among adults in developing countries are scarce. In Brazil, an annual seroconversion rate of 1.1% and a seroreversion rate of 0.2% were found during 56 months of follow-up (de Oliveira *et al.* 1999a). In India, none of the 46 endoscopically studied patients who were initially *H. pylori*-negative acquired new infection during a year (Bapat *et al.* 2000). A somewhat higher risk of the infection, a seroconversion rate of 1.9% per year, was found among missionaries and military personnel from developed countries who were residing in less developed countries (Hyams *et al.* 1995, Becker *et al.* 1999).

Generally, the infection status during adulthood is quite stable, as shown by seroepidemiological studies (Table 2) and by prospective histological cohort studies (Valle *et al.* 1996, Maaroos *et al.* 1999).

The critical period of the acquisition of the infection appears to be early childhood: the highest incidence rates have been found for children under the age of 5–7 years. The longest follow-up study over childhood, from infancy to the age of 21–23 years, was conducted in the USA (Malaty *et al.* 2002). In this study it was found that the annual incidence rate was the highest, 2.1%, at ages 4–5 years, and it decreased with advanced age. A cohort of Swedish children was monitored from infancy to 11 years of age, and the highest annual incidence of 13% was calculated for the period between 18 months and 2 years of age, while no seroconversion occurred between 4 and 11 years of age (Granström *et al.* 1997). In Finland, the annual incidence rate was found to be 1.5% during the first year of life, 3.7% during the second year of life and 0.3% between 3 and 12 years of age (Ashorn *et al.* 1996, Ashorn *et al.* 1995).

In developing countries, studies of the incidence have been mostly focused on young children, while the established rates have been high. In Ethiopia, the annual seroconversion rate was 24–31% for children aged 2–4 years (Lindkvist *et al.* 1999b). In Peru, among children with a median age of 18 months, the seroincidence rate was 12% per year (Passaro *et al.* 2001). In Bolivia, among children aged 21 months to 7 years, the annual incidence rate was the highest, 26%, in the age between 3 and 4 years (Glynn *et al.* 2002). Cross-sectional studies have also provided supporting evidence that acquisition of the infection in developing countries occurs predominantly during the first years of life, as prevalence rates exceeding 50% by the age of 5 years have often been found (Clemens *et al.* 1996, Thomas *et al.* 1999, Rocha *et al.* 2003).

High incidence can be detected in industrialized countries among children belonging to particular subgroups. In Germany, children of Turkish nationality were at a higher risk of the infection (Rothenbacher *et al.* 2000a). In the USA, the annual seroconversion rate was fourfold among black children in comparison with white children (Malaty *et al.* 1999). In a Canadian first nations community, where 95% of adults were seropositive, a cross-sectional study of children by means of faecal *H. pylori* antigen testing revealed that the infection could be acquired as early as 6 weeks of age, and that by the second year of life the prevalence of the infection was 67% (Sinha *et al.* 2002).

A number of studies have indicated that spontaneous loss of *H. pylori* infection is a common event in children (Thomas *et al.* 1999, Passaro *et al.* 2001, Perez-Perez *et al.* 2003). In a Peruvian study, the overall prevalence, determined by $^{13}$C-UBT, decreased from 71% at the age of 6 months to 48% at the age of 2 years (Klein *et al.* 1994). In an 8-year follow-up study of children and adolescents in Japan, the seroconversion and seroreversion rates were 1.1% and 1.8% per year, respectively, which resulted in the fall of the overall prevalence of the infection from 32% to 17% (Kumagai *et al.* 1998). The decrease in the seroprevalence rates, from 10% to 3% between the ages of 2 and 10 years was demonstrated also in a Swedish study (Granström *et al.* 1997). In Germany, among Turkish children aged 1 to 4 years, the annual incidence rate of the infection was 7% and the annual rate of loss of the infection was 35%,

determined by means of an antigen-based stool assay (Rothenbacher *et al.* 2002a). However, these results have to be considered with some caution owing to the possible methodological problems involved in detecting *H. pylori* infection, *e.g.* higher rate of false-positive $^{13}$C-urea breath test results in infants than in older children (Koletzko & Feydt-Schmidt 2001).

In Estonia, high seroconversion rates were found for the children's first years of life, 27% for the first year and 25% for the second year, while seroreversion rate was also high, >50% (Lindkvist *et al.* 1999a).

Little is known about the factors, which facilitate loss of the infection. The usage of antibiotics in children but not in adults has been found to be associated with loss of the infection (Rothenbacher *et al.* 1997, Rothenbacher *et al.* 2002a). On the contrary, Tindberg *et al.* (1999) did not find that antibiotic use for minor childhood morbidity was associated with the spontaneous clearance of the infection. In adults, clearance of the infection might be the result of development of gastric atrophy, *i.e.* loss of a natural niche of the organism (Valle *et al.* 1996, Kokkola *et al.* 2003).

### 4.1.3. Birth cohort effect

In developed countries, the prevalence of *H. pylori* infection increases steadily with advanced age, whereas the rate of acquisition of the infection in adulthood is low and roughly equal or even lower than the rate of loss of the infection (Table 2). Therefore, continuous increase in the prevalence of *H. pylori* with age cannot be explained by the constant recruitment of the infection throughout life. The plausible explanation is that the differences in the prevalence rates in adults born in different time periods are accounted for by the differences in the acquisition rates in their childhood, *i.e. H. pylori* infection is less prevalent in younger generations (birth cohorts) than in older ones because the risk of contracting prevalent *H. pylori* infection in childhood has substantially decreased over recent decades due to diminished acquisition, or increased loss, or both. Thus, the declining prevalence of *H. pylori* infection in younger generations in industrialized countries might be the expression of the birth cohort effect. The birth cohort effect is defined as the variation in the health status (here: the prevalence of *H. pylori* infection) that arises from different exposures to causal factors in different birth cohorts in the population as the environment and society changes (Banatvala *et al.* 1993).

To separate the birth cohort effect from the age effect, one requires not only data about the prevalence of *H. pylori* infection for one and same birth cohort at different time points, but also data for persons of the same age but from different birth cohorts. Banatvala *et al.* (1993) analyzed the sera collected in 1969, 1979 and 1989 in England, and found that age-adjusted *H. pylori* seropositivity decreased significantly over 20 years: the odds of being seropositive decreased by 26% per decade. Subsequent studies have confirmed

that the age-adjusted prevalence of *H. pylori* infection has significantly decreased in industrialized countries over time (Table 3), and that different birth cohorts have specific prevalence rates of *H. pylori* infection throughout adulthood (Vyse *et al.* 2002). It is postulated that the prevalence of the infection at age 20 provides a reasonable estimate of its prevalence in that birth cohort throughout the remainder of their lives (Graham & Graham 2002).

A similar birth cohort pattern, *i.e.* decrease in the age-adjusted incidence of diseases in successive birth cohorts, is also noted in *H. pylori* related diseases as peptic ulcer (Susser & Stein 1962, Sonnenberg 1995, Harvey *et al.* 2002), and gastric cancer (Sipponen & Kimura 1994b, Aragones *et al.* 1997, Rehnberg-Laiho *et al.* 2001).

*H. pylori* has colonized humans for thousands of years: *H. pylori* antigens have been found from the faeces of 3000-year-old mummies (Allison *et al.* 1999); molecular genetic studies carried out in different continents suggest that the microorganism was present in humans a minimum of 11 000 – 12 000 years ago (Ghose *et al.* 2002, Falush *et al.* 2003). Despite its presumed long history in humans and excellent adaptation to the host, *H. pylori* is gradually disappearing from the populations in developed countries**.** The process started several decades ago: in Sweden and Finland a drop in the age-specific prevalence of *H. pylori* infection has been noted in the birth cohorts born as early as the 1920s in comparison with the antecedent birth cohorts (Kosunen *et al.* 1997, Gause-Nilsson *et al.* 1998). Finnish data suggest that *cagA*-positive *H. pylori* strains are disappearing faster than *cagA*-negative strains (Perez-Perez *et al.* 2002). Mathematical modelling of prevalence trends has indicated that the transmissibility of *H. pylori* has decreased to values so low that the organism will totally disappear from the populations of the industrialized countries, however, this process will take more than a century without targeted intervention (Rupnow *et al.* 2000).

Smaller family size, less crowded living conditions, improved sanitation and clean water supply, in addition to the higher likelihood of antibiotic administration to children, are implicated as the potential reasons for the decline in *H. pylori* incidence and prevalence in industrialized countries (Go 2002). Interestingly, in Eastern European countries, such as in Estonia, Poland, and Russia, despite the small number of children in families, apparently better sanitation than in developing countries and access to antibiotics for several decades, the prevalence of the infection has been found to be very high, >80% in all age groups in adulthood including young adults (Vorobjova *et al.* 1994, Matysiak-Budnik *et al.* 1996, Malaty *et al.* 1996a). Since the beginning of the 1990s, rapid radical changes towards westernization have occurred in these societies. The issue whether the prevalence of *H. pylori* infection undergoes changes in such circumstances has not yet been studied and was addressed in the present study.

**Table 3.** Changes in the age-adjusted prevalence rates of *H. pylori* infection over time

| Reference | Country | Study population | Duration of the study | Age-adjusted prevalence rate (%) | |
|---|---|---|---|---|---|
| | | | | at the beginning of the study | at the end of the study |
| Sipponen *et al.* 1994a | Finland | gastroscopied patients, aged 20–49 years | 15 years (1977–1992) | 66[a] | 41[a] |
| Roosendaal *et al.* 1997 | Netherlands | children with viral respiratory infections | 15 years (1978–1993) | 19[b]<br>23[c] | 9[b]<br>11[c] |
| Kosunen *et al.* 1997 | Finland | randomly selected 15–74-year-old persons | 21 years (1973–1994) | 56 | 31 |
| Haruma *et al.* 1997 | Japan | gastroscopied adults | 16 years (1975/78–1991/94) | 55 | 29 |
| Gause-Nilsson *et al.* 1998 | Sweden | random sample of 70-year-old persons | 21 years (1971–1992) | 78 | 52 |
| Fujisawa *et al.* 1999 | Japan | participants of health screening programmes, aged 0–89 years | 20 years (1974–1994) | 73 | 39 |
| Rehnberg-Laiho *et al.* 2001 | Finland | pregnant women, aged 20–34 years | 22 years (1983–1995) | 30 | 13 |
| Apostolo-poulos *et al.* 2002 | Greece | donors and outpatients, aged 15–85 years | 10 years (1987–1997) | 60 | 49 |

[a] prevalence of chronic gastritis; 94% of non-atrophic and 50% of atrophic gastritis cases were *H. pylori* positive
[b] at age 6–8 years
[c] at age 12–15 years

### 4.1.4. Transmission of *H. pylori* infection

The exact routes of transmission are not definitely known due to the inability to clinically detect acute *H. pylori* infection as well as due to technical difficulties in isolating the microorganism from the sources other than the gastric mucosa. Transmission of the infection probably occurs in multiple pathways, which may differ in different societies and age groups. As childhood is a period of high risk for *H. pylori* acquisition, a good understanding of the mode(s) of transmission

in children is required to identify how to break the chain of transmission of the infection.

The minimum infectious dose of *H. pylori* for humans is not yet established. In human volunteers, ingestion of $10^4$–$10^{10}$ of *H. pylori* after administration of famotidine resulted in infection in 18 out of 20 subjects (Graham *et al.* 2004). For non-human primates, the established minimum infectious dose of *H. pylori* is $10^4$ cfu (Solnick *et al.* 2001).

The most important reservoir of *H. pylori* is the human stomach; and potentially, *H. pylori* may pass from the stomach into the external environment by faeces, vomitus or gastric regurgitation.

#### 4.1.4.1. Faecal-oral and gastro-oral routes

For faecal-oral transmission, *H. pylori* must be excreted by faeces, viable and at sufficiently high concentrations. Culturing of *H. pylori* from normal faeces has been rarely successful (Leverstein-van Hall *et al.* 1993), although higher isolation rates have obtained recently by using modified culturing conditions (Dore *et al.* 2000, Liang & Redlinger 2003). *H. pylori* can be more easily isolated from diarrheal stool (Thomas *et al.* 1992, Parsonnet *et al.* 1999, Haggerty *et al.* 2003) indicating that *H. pylori* may preserve its viability better if the transit time through the gastrointestinal tract is shorter. The number of bacteria isolated in diarrheal stool, though, is relatively small, 5–2125 cfu/ml (Parsonnet *et al.* 1999). The shedding of a large number of viable *H. pylori*, up to 30 000 cfu/ml has found in artificially induced vomitus, and *H. pylori* has been isolated from the air sampled in the vicinity of the vomitus (Parsonnet *et al.* 1999). *H. pylori* has been successfully cultured also from naturally secreting vomitus in a child with acute gastroenteritis (Leung *et al.* 1999). This proves that the organism is potentially transmissible during episodes of gastrointestinal tract illness, particularly with vomiting (Parsonnet *et al.* 1999). History of vomiting in siblings was found to be an independent risk factor for *H. pylori* infection in children (Luzza *et al.* 2000). Laporte *et al.* (2004) followed prospectively neurologically handicapped children living in an institution, and found a chronological link between outbreaks of gastroenteritis and new cases of *H. pylori* infection. Therefore, the decreased incidence of diarrheal diseases, parallel with socioeconomic development, is one of the possible explanations for the decreased incidence of *H. pylori* infection.

The iatrogenic transmission of *H. pylori* from stomach to stomach *via* contaminated endoscopic devices is possible but rare event (Langenberg *et al.* 1990). Proper disinfection of endoscopes prevents iatrogenic spread (Cronmiller *et al.* 1999).

Hands may serve as a potential vector of the infection. In rural Guatemala, carriage of *H. pylori* under fingernail was detected in 58% of studied persons using PCR method (Dowsett *et al.* 1999).

#### 4.1.4.2. Oro-oral route

Human oral cavity has been implicated as a possible source of *H. pylori* infection, although there exists controversy as to whether the oral cavity can act as a permanent reservoir of *H. pylori,* or whether the microorganism can be detected only occasionally in the saliva or on the oral mucosal surfaces as result of gastroesophageal reflux or vomiting. The fastidious nature of *H. pylori* and the complexity of the oral microflora make the isolation of the microorganism from the oral cavity complicated. Most reports about presence of *H. pylori* in the oral cavity are based on detection of a specific DNA, which has been found in the dental plaque (Nguyen *et al.* 1993, Oshowo *et al.* 1998, Song *et al.* 2000a), in the periodontal pockets (Dowsett *et al.* 1999), and in the saliva (Li *et al.* 1996, Song *et al.* 2000a). The detection rate, though, has shown a great deal of variation, from less than 10% among the subjects harboring the organism in the stomach (Oshowo *et al.* 1998) to 100% among the subjects under study, irrespective their gastric *H. pylori*-status (Song *et al.* 2000a). The possibility of misidentification due to the complex and rich oral microflora cannot be excluded. A major weakness of PCR is its inability to distinguish between viable or dead microorganisms, and therefore, detection of the DNA of the microorganism in the oral cavity is not sufficient evidence for considering it a reservoir of the infection. Although successful culture of *H. pylori* from the oral cavity has also been reported, the success rate is low (Krajden *et al.* 1989, Ferguson *et al.* 1993, Oshowo *et al.* 1998, Parsonnet *et al.* 1999). The number of organisms in the oral cavity, if present, is rather small: using a competitive PCR assay the median number of *H. pylori* in dental plaque of adults with gastric *H. pylori* infection was found to be 25 cells/mg (Song 2000b); in the postemesis saliva collected half an hour after vomiting *H. pylori* cultures had counts ranging from 50–500 cfu/ml (Parsonnet *et al.* 1999).

Some epidemiological results also favor spread of *H. pylori* by an oral source. Premastication of food and plate sharing were found to be independent risk factors for *H. pylori* infection of children (Albeneque *et al.* 1990, Nabwera *et al.* 2000).

For children, the oral-oral transmission route, from caretaker-to-child, through licking pacifiers or tasting the children's food, or through some other practices, as well as from child-to-child, owing to small children's habit contact the things orally, seems plausible.

#### 4.1.4.3. Environmental sources: water and food

The analysis of the genome has shown that it is very unlikely that *H. pylori* can multiply in environment (Tomb *et al.* 1997, Lee 1998).

*H. pylori* can survive in water, milk and in various foods under refrigerated storage for several days, suggesting that the water or food contaminated with

*H. pylori* could be potentially infectious to humans (Fan *et al.* 1998, Stevenson *et al.* 2000, Poms & Tatini 2001). In water, *H. pylori* remained culturable for up to 24 hours at 20–23ºC and for 2–3 days at 16º C (Adams *et al.* 2003). *H. pylori* has not been cultured from natural freshwater, but was isolated from wastewater in Mexico (Lu *et al.* 2002). Using PCR method, *H. pylori* DNA has been detected in drinking water (Hulten *et al.* 1996, McKeown *et al.* 1999**,** Horiuchi *et al.* 2001, Krumbiegel *et al.* 2004). *H. pylori* DNA has been detected also in biofilms within water storage pots or water distribution systems (Bunn *et al.* 2002, Watson *et al.* 2004). Few quantitative data are available. Krumbiegel *et al* (2004) found that the *H. pylori* DNA was present in approximately one tenth of the private wells in rural counties in Germany, and that estimated average infestation was approximately one *H. pylori* cell per ml. However, a positive PCR result does not prove that the organism is viable and transmissible.

Under unfavorable conditions, *H. pylori* may transform from an actively dividing spiral-shaped form into a nonculturable coccoid form (Catrenich & Makin 1991), which may represent an alternative survival system (Nilsson *et al.* 2002, Adams *et al.* 2003) or a morphologic manifestation of cell death (Kusters *et al.* 1997). These coccoid forms may persist for extended periods in water (Fan *et al.* 1998, Adams *et al.* 2003). The question whether the coccoid form of *H. pylori* is able to establish infection in humans is still unclear. In laboratory conditions, coccoid *H. pylori* organisms given at high dosage to mice were able to colonize their gastric mucosa and cause inflammation (She *et al.* 2001).

In epidemiological studies, generally no association has been found between *H. pylori* infection and a water source in industrialized countries, possibly because of high quality water treatment (Fiedorek *et al.* 1991, Elitsur *et al.* 1998, Yamashita *et al.* 2001). Water-borne transmission may occur in regions of the world where the quality of drinking water is poor (Klein *et al.* 1991, Bunn *et al.* 2002, Nurgalieva *et al.* 2002). Stored household water, contaminated in home, may also serve as a vehicle of the infection (Glynn *et al.* 2002).

#### 4.1.4.4. Animal reservoirs

Animals are unlikely to be an important reservoir of *H. pylori* infection (Graham *et al.* 1991, Ansorg *et al.* 1995, Staat *et al.* 1996, Bode *et al.* 1998a, Brown *et al.* 2001), although in specific settings, zoonotic transmission may occur.

Sheep have been reported to harbor *H. pylori* in their stomachs (Dore *et al.* 2001). *H. pylori* was isolated from sheep's milk (Dore *et al.* 1999a), suggesting that it might be a transmission vehicle of *H. pylori.* Regular professional contact with sheep results in almost 100% *H. pylori* prevalence: 98% of studied shepherds in Italy and in Poland were seropositive (Dore *et al.* 1999b, Papiez *et*

*al.* 2003). Children who had contact with sheep also had increased prevalence odds of the infection (Goodman *et al.* 1996).

The role of insects as a potential vector has been studied as well. Houseflies and cockroaches, when fed pure cultures of *H. pylori*, were able to harbor the microorganisms in their gut and excreta for more than 24 hours after initial exposure (Grübel *et al.* 1997, Imamura *et al.* 2003). However, *H. pylori* was not recovered from any of the houseflies fed human faeces either naturally infected or artificially infected with *H. pylori*, thereby not confirming that houseflies are vectors for transmission (Osato *et al.* 1998).

#### 4.1.4.5. Intrafamilial transmission

*H. pylori* infection clusters within families: children living with infected parents have higher prevalence of the infection than those living with uninfected parents (Drumm *et al.* 1990, Malaty *et al.* 1991, Dominici *et al.* 2000, Zhou *et al.* 2000). Molecular studies have shown that family members often share the same strain of *H. pylori* (Bamford *et al.* 1993, Roma-Giannikou *et al.* 2003, Kivi *et al.* 2003). Intrafamilial clustering of the infection suggests either a person-to-person transmission within family or exposure to a common environmental source of the infection. Close intrapersonal contact appears to facilitate spread of the infection: domestic overcrowding in childhood has been consistently found to be a significant risk factor for *H. pylori* infection both for current children (Mitchell *et al.* 1992a, Malaty *et al.* 1996a, McCallion *et al.* 1996, Wizla-Derambure *et al.* 2001, Rodrigues *et al.* 2004) as well for adults (Mendall *et al.* 1992, Webb *et al.* 1994, Malaty *et al.* 1994, Malaty *et al.* 1998), which supports the theory of person-to-person transmission.

Who in the family plays the key role in transmission of the infection to the child might depend on the setting. In developing countries, where there are large families with many siblings, sib-sib transmission might be more important (Goodman & Correa 2000, Glynn *et al.* 2002). In developed countries, where the number of children in families is generally small, mothers may play the key role (Rothenbacher 1999, Malaty *et al.* 2000, Rothenbacher *et al.* 2002b). Studies have generally failed to identify *H. pylori*–positive father as a risk factor of the infection in offspring (Miyaji *et al.* 2000, Rocha *et al.* 2003). Kivi *et al.* (2003) analyzed the *H. pylori* strains of 39 families by means of DNA fingerprinting but did not find strain concordance between the fathers and the offspring, whereas the siblings as well as the mothers and the offspring commonly shared strains. Child-to-adult transmission seems to be an unlikely event. During a nine-year follow-up of 46 families in Japan no case was identified when the infection spread from an infected child to an uninfected parent (Malaty *et al.* 2000).

Outside family, child-to-child transmission does not seem to be a route of major importance in Western societies. In a study conducted in France, the

attendance of a nursery or school before the age of 6 years was not associated with infection, whereas the number of persons at home was strongly associated with *H. pylori*-positivity in children (Wizla-Derambure *et al.* 2001). In Sweden, no increased risk for *H. pylori* infection was seen among children who had attended day-care centers in comparison with those who had been exclusively looked after at home (Tindberg *et al.* 2001a).

## 4.2. Characterization of the microorganism

*H. pylori* is a spiral-shaped, Gram-negative, microaerophilic flagellated bacterium, which colonizes the human stomach. The *H. pylori* genome is relatively small, in average 1.67 million basepares (Tomb *et al.* 1997). Genome analysis has shown that *H. pylori* has few regulatory pathways, which indicates that *H. pylori* is adapted to colonize only a restricted ecologic niche (Tomb *et al.* 1997, Lee 1998). Within its ecologic niche, the human stomach, *H. pylori* is extremely well adapted: once acquired, the infection generally persists for the lifetime of the host. *H. pylori* produces urease, which hydrolyses urea to ammonia, neutralizing the microenvironment of its immediate surroundings within the stomach (Marshall & Langton 1986). Its helicoidal shape and high motilility due to action of multiple flagella allow it to cross the thick layer of mucus and stay beneath it, thereby making the successful colonization of the human stomach possible, where gastric acidity and peristalsis normally inhibit bacterial colonization (Montecucco & Rappuoli 2001). Most of the *H. pylori* organisms are free-living in the gastric mucus, but *H. pylori* is able to strongly adhere to gastric epithelial cells *via* interaction between bacterial adhesins and host receptors (Ilver *et al.* 1998).

The important virulence factors of *H. pylori* are the vacuolating cytotoxin (VacA), encoded by the *vacA* gene, and the cytotoxin associated protein (CagA), encoded by the *cagA* gene, which is part of the *cag* pathogenicity island (*cag* PAI). The VacA induces epithelial cell vacuolization (Leunk *et al.* 1988), forms channels in epithelial cell membranes, thereby providing the bacterium with nutrients (Szabo *et al.*1999), and acts on host mitochondria (Galmiche *et al.* 2000). The *cag* PAI is a fragment of DNA containing the genes encoding a type IV secretion system that injects bacterial proteins into host cells (Censini *et al.* 1996). After attachment of *cag* PAI-positive *H. pylori* cells to gastric epithelia, CagA is injected from the bacteria into the host cells, where phosporylated CagA leads to reorganization of the actin cytoskeleton and functional alterations of the gastric epithelial cells (Segal *et al.* 1999, Stein *et al.* 2000, Higashi *et al.* 2002, Yamazaki *et al.* 2003). *H. pylori* strains show a high grade of genetic diversity (Go *et al.* 1996) and broad geographic variation of genotypes (van Doorn *et al.* 1999). In East Asia nearly all *H. pylori* strains but

in the other parts of the world approximately 50–70% of them are *cagA*-positive (Shimoyama *et al.* 1997, Parsonnet *et al.* 1997, Vorobjova *et al.* 1998, Yang *et al.* 1999). The *vacA* gene is present in essentially all strains, but only approximately half of the *H. pylori* strains produce VacA (Atherton *et al.* 1995). This phenomenon is due to *vacA* polymorphism, the two most diverse regions being the signal region (which can be type s1 or s2) and the mid-region (which can be type m1 or m2). The strains with the *vacA* s1m1 alleles exhibit the highest vacuolating cytotoxin activity, and the strains with the *vacA* s2m2 alleles have low or zero cytotoxin activity (Atherton *et al.* 1995, Letley *et al.* 2003). Although *cag* PAI and *vacA* are far apart in the *H. pylori* chromosome (Tomb *et al.* 1997), presence of *cag* PAI is closely associated with presence of *vacA* type s1 and absence of *cagA* with presence of the *vacA* type s2 (Atherton *et al.* 1995).

In many regions of the world, infection with *H. pylori cagA*- and *vacA* s1-positive genotypes have been found to be associated with higher risk for peptic ulcer (Covacci *et al.* 1993, Atherton *et al.* 1995, van Doorn *et al.* 1999), atrophic gastritis (Kuipers *et al.* 1995, Maaroos *et al.* 1999) and gastric cancer (Huang *et al.* 2003). However, no microbial factors that reliably predict the clinical outcome of *H. pylori* infection, have yet been identified.

## 4.3. Clinical manifestations of *H. pylori* infection in children

### 4.3.1. Natural course of *H. pylori* infection

Acute *H. pylori* infection in adults is accompanied by mild to moderate dyspeptic symptoms and occasional vomiting, which appear few days after challenge, peak during the second week and then resolve (Marshall *et al.* 1985, Morris *et al.* 1987, Graham *et al.* 2004). In children, the symptomatology of acute *H. pylori* infection is not well characterized. Occasionally acute infection may cause gastric ulcers and hematemesis (Mitchell *et al.* 1992b). After acquisition of the infection, development of transient gastric hypochlorhydria is common, which in most subjects resolves within months (Morris *et al.* 1987, Harford *et al.* 2000).

The clinical course of chronic *H. pylori* infection is highly variable and influenced by microbial, host and environmental factors (El-Omar *et al.* 2000, Höcker & Hohenberger 2003). In virtually all infected individuals *H. pylori* causes chronic inflammation in the gastric mucosa (Dooley *et al.* 1989, Maaroos *et al.* 1991b, Macarthur *et al.* 1995). Gastritis develops rapidly after acquisition of *H. pylori* infection (Graham *et al.* 2004) and persists along with its persistence (Valle *et al.* 1996, Maaroos *et al.* 1999). Through several decades

of the infection, chronic gastritis may gradually progress to atrophic gastritis. The annual incidence of atrophic gastritis among *H. pylori*-positive adults is approximately 1–3% (Kuipers 1998, Maaroos *et al.* 1999). Most people with *H. pylori* infection are asymptomatic, but a proportion of infected individuals develop severe gastroduodenal disease, including duodenal ulcer, gastric ulcer, gastric adenocarcinoma, and gastric MALT lymphoma (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 1994, Parsonnet 1994). Patients with antrum-predominant gastritis are predisposed to duodenal ulcers, while patients with corpus-predominant gastritis are more likely to have gastric ulcers, gastric atrophy and ultimately gastric carcinoma (Suerbaum & Michetti 2002). *H. pylori* infected persons have approximately a 4–6-fold increased risk of developing peptic ulcer disease (Nomura *et al.* 1994, Brenner *et al.* 1998, Rosenstock *et al.* 2003) and approximately a 6-fold increased risk of non-cardia gastric adenocarcinoma and gastric MALT lymphoma (*Helicobacter* and Cancer Collaborative Group 2001, Parsonnet *et al.* 1994) compared with uninfected persons. In *H. pylori*-infected subjects, the estimated lifetime risk of peptic ulcer is 6–20% (Feldman *et al.* 1998); the estimated lifetime risk of gastric cancer is 1–2% in Western societies (Kuipers 1998) and 11–12% or even higher in Japan (Uemura *et al.* 2001, Graham & Graham 2002).

It is hypothesized that early life acquisition of *H. pylori* may increase the risk of subsequent development of gastric cancer (Blaser *et al.* 1995). In an animal experiment with Mongolian gerbils, early acquisition of *H. pylori* significantly increased their susceptibility to gastric chemical carcinogenesis as compared with the case of later infection (Cao *et al.* 2002).

### 4.3.2. Chronic gastritis and peptic ulcer disease

Majority of children infected with *H. pylori* develop chronic gastritis (Drumm *et al.* 1987, Maaroos *et al.* 1991b, Macarthur *et al.* 1995). Occasionally, *H. pylori* can be found in the normal gastric mucosa in children (Maaroos *et al.* 1991b, Gottrand *et al.* 1997). A characteristic endoscopic finding of *H. pylori*–associated gastritis in children is antral nodularity which is reported to be present in 44–76% of endoscopically investigated *H. pylori*-positive children (Prieto *et al.* 1992, Luzza *et al.* 2001, Uhlig *et al.* 2003) but rarely in adults (Miyamoto *et al.* 2003). Histologically, antral nodularity is associated with presence of lymphoid follicles and higher grades of gastric inflammation (Prieto *et al.* 1992, Luzza *et al.* 2001, Uhlig *et al.* 2003), and it is found more commonly in children with duodenal ulcer (Kato *et al.* 2004). In the course of time, progressive inflammatory changes occur in the gastric mucosa of *H. pylori*-positive children (Ganga-Zandzou *et al.* 1999), although atrophic gastritis and intestinal metaplasia are found extremely rarely (Maaroos *et al.* 1991b, Meining *et al.* 1996, Uhlig *et al.* 2003). Bedoya *et al.* (2003) compared the histopathology of the gastric mucosa of *H. pylori* infected children from a

population at high risk for gastric cancer with that of a lower-risk population, and found that children from the higher-risk population exhibited more severe polymorphonuclear neutrophil and lymphocyte infiltration, mucus depletion, and *H. pylori* colonization density.

Peptic ulcer is an uncommon disease in children, and its incidence is not known. Peptic ulcer is even rare among children with abdominal complaints. Roma *et al.* (2001) reviewed the data of 2550 children studied by upper gastrointestinal endoscopy over a period of 9 years, and found that only 52 (2%) of them had peptic ulcer. Large pediatric gastroenterology centers in Canada have reported less than 10 peptic ulcer cases per year (Drumm *et al.* 1988, Israel & Hassall 1993). The incidence is presumably higher in high *H. pylori*-prevalence settings; for example, in Ufa, Russia, duodenal ulcer was diagnosed in 16% of 225 consecutively gastroscopically studied children during a 10-month period (Nijevitch & Shcherbakov 2004)**.**

As in adults, in children majority of duodenal ulcers are associated with *H. pylori* infection (Macarthur *et al.* 1995). The 94–100% prevalence of *H. pylori* infection in children with duodenal ulcer has been reported in Poland, Ireland and Russia (Bak-Romaniszyn *et al.* 1996, Goggin *et al.* 1998, Nijevitch & Shcherbakov 2004), 83% from Japan (Kato *et al.* 2004), and 62% from Greece (Roma *et al.* 2001). The natural history of duodenal ulcer in children is very similar to that reported for adults. Without *H. pylori* eradication, duodenal ulcer represents a chronic relapsing condition (Murphy 1987, Drumm *et al.* 1988, Israel & Hassall 1993), while eradication of *H. pylori* cures the disease (Israel & Hassall 1993, Goggin *et al.* 1998, Roma *et al.* 2001).

The reports on association between gastric ulcer and *H. pylori* infection in children include a small number of patients, and *H. pylori* is found less than 50% of cases (Macarthur *et al.* 1995, Roma *et al.* 2001, Kato *et al.* 2004).

### 4.3.3. Recurrent abdominal pain

According to Apley's criteria, recurrent abdominal pain (RAP) is defined as at least three episodes of abdominal pain severe enough to affect the child's activities and occurring over a period not less than three months (Apley & Naish, 1958a). RAP is a common problem: the prevalence among school-children is 9–16% (Apley & Naish 1958a, Øster 1972, Boey & Goh 2001, De Giacomo *et al.* 2002, Kokkonen *et al.* 2004). RAP is a heterogeneous condition. Initially, less than 10% of affected children were found to have a defined cause of their pain, mostly inflammatory bowel disease, peptic ulcer or urinary tract diseases (Apley 1958b, Stickler & Murphy 1979). Advances in medical diagnostics have led to an increase in the identification of specific disorders, such as lactose intolerance, milk protein intolerance and gastro-oesophageal reflux disease (van der Meer *et al.* 1992a**,** Størdal *et al.* 2001, Kokkonen *et al.* 2004).

However, in majority of children with RAP, no specific cause for the syndrome could be identified.

The discovery of *H. pylori* lead to the question whether *H. pylori*–associated gastritis without concomitant peptic ulcer might be the cause of RAP in children, and the issue is still controversial.

Nowadays only a small minority, less than 10% of children having RAP are infected with *H. pylori* in many developed countries **(**Mavromichalis *et al.* 1992**,** van der Meer *et al.* 1992b, O'Donohoe *et al.* 1996, Macarthur *et al.* 1999, Størdal *et al.* 2001, Kokkonen *et al.* 2004). Generally, community-based studies have not established that *H. pylori* infection is over-represented among children with RAP (O'Donohoe *et al.* 1996, Bode *et al.* 1998b, Macarthur *et al.* 1999, Bode *et al.* 2003). However, in some studies, *H. pylori* infection was found to be more prevalent in children with recurrent abdominal pain (Camorlinga-Ponce *et al.* 1998, De Giacomo *et al.* 2002)

*H. pylori* infection was not found to be associated with specific clinical symptomatology in most studies (Reifen *et al.* 1994, Blecker *et al.* 1996, Bode *et al.* 1998b, Roma *et al.* 1999). Epigastric pain, nocturnal pain, and episodic abdominal pain were found to be predictors of peptic ulceration rather than of *H. pylori* infection (Sherman & Macarthur 2001).

The issue whether eradication of *H. pylori* infection results in resolution of abdominal symptoms in children with chronic gastritis is also controversial, due to the lack of randomized placebo-controlled trials with sufficient power. Some studies claimed that a symptomatic relief has occurred after treatment of *H. pylori* infection. However, these studies have severe methodological limitations: none of them was placebo controlled, and often no discrimination between those *H. pylori* eradication occurred and those who remained *H. pylori* positive was made (Heldenberg *et al.* 1995, Kimia *et al.* 2000, Frank *et al.* 2000, Uc & Chong 2002). Yet some studies have found that *H. pylori* eradication led to more sustained improvement of symptoms compared with no-eradication cases (Özen *et al.* 2001, Oderda *et al.* 2004). In contrast, a number of studies conducted in children with RAP without concomitant peptic ulcer have not found any association between *H. pylori* eradication and resolution of symptoms (Ashorn *et al.* 1994, Wewer *et al.* 2001, Levine *et al.* 2004). To the best of my knowledge, only one double-blind randomized placebo-controlled *H. pylori* eradication trial in children with RAP has been published, which concluded that healing of gastric inflammation does not lead to symptomatic relief in children (Ashorn *et al.* 2004). However, the study might be underpowered to detect differences, as altogether only 20 patients were involved.

### 4.3.4. Extragastric manifestations of *H. pylori* infection

Associations have been found between *H. pylori* infection and iron-deficiency anemia. Although a number of studies have explored the association of *H. pylori* infection with atopic disorders, acute infectious diarrhea and diminished growth, the results are not conclusive. As associations between other various childhood disorders, as SIDS, epilepsy, migraine, *etc.* and *H. pylori* infection have often been established on the basis of small series, the results might have been confounded by other factors, or be merely accidental, while these associations have not been found consistently.

#### 4.3.4.1. Iron-deficiency anemia

*H. pylori*–infected subjects have lower mean serum ferritin levels compared with the non-infected ones irrespective to their iron intake (Milman *et al.* 1998, Berg *et al.* 2001). *H. pylori* infection is more prevalent among subjects with low serum ferritin level than among subjects with normal serum ferritin level, both in the case of adults and children (Milman *et al.* 1998, Parkinson *et al.* 2000, Berg *et al.* 2001, Seo *et al.* 2002a). A number of case reports and case series have described resolution of refractory iron deficiency anemia only after eradication of *H. pylori* infection, thereby supporting the hypothesis that *H. pylori* infection causes iron deficiency and not *vice versa* (Dufour *et al.* 1993, Annibale *et al.* 1999, Ashorn *et al.* 2001, Russo-Mancuso *et al.* 2003, Hacihanefioglu *et al.* 2004). Choe *et al.* (1999) conducted a double-blind placebo-controlled trial in children and adolescents with iron deficiency anemia and coexisting *H. pylori* infection, and demonstrated that *H. pylori* eradication with or without iron supplementation led to more rapid resolution of anemia compared with mere iron administration only.

The mechanisms through which *H. pylori* infection can cause iron deficiency and further lead to anemia have not been fully elucidated. It has been suggested that *H. pylori* infection may increase the iron demand as *H. pylori* itself uses iron for its growth and may capture ingested iron and iron from human lactoferrin (Velayudhan *et al.* 2000, Ciacci *et al.* 2004). Bleedings from gastrointestinal tract, due to peptic ulcer or atypical hemorrhagic gastritis may also contribute to iron deficiency (Yip *et al.* 1997). Majority of *H. pylori*-positive persons do not develop anemia. It is possible that presence of specific bacterium strains and the high need for iron required by the host under particular conditions could both play a crucial role in development of iron-deficiency anemia (Choe *et al.* 2001, Jeon *et al.* 2004).

#### 4.3.4.2. Atopic diseases

The prevalence of atopic diseases has increased markedly in recent decades in the Western countries, but not to such an extent in the Eastern European countries, while the differences in prevalence of atopic diseases between Eastern and Western Europe have been found to be largely limited to the populations born after the late 1950s, at a time when the lifestyle of the two parts of the continent began to drift apart (von Hertzen & Haahtela 2004). The composition of the microflora of the gastrointestinal tract may play a role in development of and protection from allergy (Björksten *et al.* 2001). The increase in the occurrence of atopic diseases appears to have coincided with the decrease in the prevalence of *H. pylori* infection. Studies that have explored the possible association between *H. pylori* infection and atopic diseases have yielded conflicting results. In some studies, which have addressed predominantly respiratory allergy, decreased prevalence of atopic diseases and/or allergen-specific IgE antibodies has been found among *H. pylori*-positive adults (Kosunen *et al.* 2002, McCune *et al.* 2003). It cannot be excluded that instead of having a protective effect against atopic diseases as a single agent *per se, H. pylori* infection is only a marker for a lifestyle with high exposure to microbials, while this lifestyle is protective (Linneberg *et al.* 2003). Some studies have found no associations between *H. pylori* infection and atopic diseases (Uter *et al.* 2003, Cullinan *et al.* 2003), whereas some pediatric case-control studies point out that *H. pylori* infection, especially the infection with a CagA-positive strain may actually increase the risk of food allergy (Corrado *et al.* 1998, Figura *et al.* 1999). Accordant with these results, the animal experiments and *in vitro* studies have demonstrated that *H. pylori* infection increases the transcellular passage of macromolecules and inhibits development of oral tolerance to food antigens, thereby suggesting that *H. pylori* infection may predispose to food allergy (Matysiak-Budnik *et al.* 2003, Matysiak-Budnik *et al.* 2004).

#### 4.3.4.3. Acute intestinal infections

Some studies have suggested that *H. pylori* infection may predispose to various gastrointestinal infections as cholera (Clemens *et al.* 1995) and shigellosis (Shmuely *et al.* 2004). Newly acquired *H. pylori* infection in children was followed by increased occurrence of diarrhea (Passaro *et al.* 2001). Transient hypochlorhydria has thought to be the mechanism underlying this phenomenon. Passaro *et al.* (2001) postulated that *H. pylori*-promoted gastroenteritis some months after acute infection is a biologically plausible mechanism for successful spread of the *H. pylori* infection. In contrast, in other studies, *H. pylori* infected children and adults had significantly less acute diarrheal illnesses than non-infected subjects (Rothenbacher *et al.* 2000b, Bode *et al.*

2001, Perry *et al.* 2004). It cannot be excluded that some confounding factors may also explain the conflicting results of these observational studies.

#### 4.3.4.4. Diminished growth

Several population-based cross-sectional studies, conducted both in developing as well as developed countries, have found an association between diminished growth and *H. pylori* infection in children (Patel *et al.* 1994, Perri *et al.* 1997, Richter *et al.* 2001), whereas others have not found such association (Clemens *et al.* 1996, Oderda *et al.* 1998, Quinonez *et al.* 1999). It is possible that *H. pylori* infection is merely a marker for other factors causing growth retardation. Oderda *et al.* (1998) did not find that *H. pylori* infection was a risk factor for short stature in children after controlling for the socioeconomic status of families. Choe *et al.* (2000) found that the mean height of schoolchildren was significantly lower in the group having both *H. pylori* infection and iron deficiency anemia but not in the group with *H. pylori* infection without anemia in comparison with *H. pylori*-negative children.

However, a temporal relationship between acquisition of the infection and subsequent growth impairment was demonstrated in some prospective studies. Seroconversion was found to be associated with the slowing of weight gain but only among those aged 2 years and older (Passaro *et al.* 2002). Another study conducted among children aged 1–5 years demonstrated that a new and sustained infection was followed by significant growth retardation (Bravo *et al.* 2003). Additional evidence would come from interventional studies, however, to the best of my knowledge, no studies have been published yet on the effect of eradication of *H. pylori* infection on growth in children.

## 4.4. Diagnostic tests for detection of *H. pylori* infection

The diagnostic tests for *H. pylori* infection can be roughly divided into two categories: biopsy-based tests which are invasive tests because as they require gastroscopy, and non-invasive or minimally invasive tests where no gastrocsopy is required and the serum, whole blood, faeces, expired air, saliva or urine are used for testing.

Selection of the test depends on the purpose of testing, sensitivity and specificity of the test, cost-effectiveness of testing strategy and availability of the test. For clinical purposes, in children gastroscopy and biopsy-based tests for *H. pylori* infection are preferred, as children should be investigated for *H. pylori* infection only if they present with symptoms, which are suggestive for organic disease (Sherman *et al.* 1999, Drumm *et al.* 2000, Gold *et al.* 2000). In

the case of epidemiological investigations, biopsy-based tests should not be used for ethical reasons.

### 4.4.1. Biopsy-based tests

Biopsy-based tests include histological examination, culture, rapid urease test and tests based on molecular methods.

***Histological examination***. *H. pylori* can be directly visualized by histological examination on the gastric mucosa as characteristic spiral or curve-shaped bacteria. This allows estimation of *H. pylori* colonization density as well as provides permanent record. For optimal assessment and for minimizing sampling error, at least two biopsy samples from both the gastric antrum and corpus mucosa should be taken (Dixon *et al.* 1996). The Giemsa stain appears to be the preferred stain for evaluation of *H. pylori* infection on the basis of its good sensitivity, excellent specificity, and lack of technical difficulty in preparation (Laine *et al.* 1997, Rotimi *et al.* 2000). Besides diagnosing *H. pylori* infection, histology has the advantage of allowing the excellent visualization of morphological changes in the gastric mucosa; for the latter purpose, hematoxylin and eosin stain are generally used. The results of histological examination are somewhat dependent upon experience and on the accuracy of the pathologist, but generally, histological examination has high sensitivity and specificity for detecting *H. pylori* infection, with high interobserver agreement (Thijs 1996, el-Zimaity *et al.* 1996, Chen *et al.* 1999).

***Culture*** is a very specific method for diagnosing *H. pylori* infection, however, culturing is technically demanding and time-consuming, and its sensitivity varies among laboratories (Zullo *et al.* 2003). In experienced laboratories, sensitivity higher than 90% is achieved (Mégraud *et al.* 1999). The benefit of culture is the possibility to determine the susceptibility of the strain to antimicrobial agents.

***Rapid urease test*** is based on the capacity of *H. pylori* to secrete the enzyme urease. When *H. pylori* infected biopsy specimen is placed in a urea-containing medium, the bacterial urease splits urea into ammonia and $CO_2$, and pH of the medium rises. This is detectable by the color change of the pH indicator dye. It is a quick and simple test with a sensitivity about 88–90% and a specificity close to 100% (Malfertheiner *et al.* 1996). The sensitivity of the test is lower in patients with active or recent upper gastrointestinal bleeding (Lee *et al.* 2000). The test indicates only the presence or absence of the infection and gives no additional information.

***Tests based on molecular methods***, mainly the PCR are used in order to detect *H. pylori* from biopsy specimens with high sensitivity and specificity (Weiss *et al.* 1994, Fabre *et al.* 1994), to detect point mutations encoding *H. pylori* resistance to antibiotics, especially to clarithromycin (Matsumura *et*

*al.* 2001, Menard *et al.* 2002), and to detect virulence factors (Ruzsovics *et al.* 2004).

### 4.4.2. Tests based on detection of specific anti-*H. pylori* antibodies

*H. pylori* infection induces a humoral immune response in the host, resulting in an early increase of specific IgM antibodies, and later a persistent increase of specific IgG and IgA antibodies (Gold *et al.* 1997). Specific antibodies can be detected in serum, whole blood, urine and saliva, both from fresh and freeze-stored samples. IgG-based tests have higher accuracy than IgA-based tests (Karvar *et al.* 1997, Kindermann *et al.* 2001).

The limitation of the tests based on specific antibody detection is that acute infection can be missed due to the delay in antibody production as IgG seroconversion occurs approximately 7–14 weeks after infection (Frommer *et al.* 1988, Morris *et al.* 1991, Sobala *et al.* 1991). On the other hand, false-positive results may occur, as antibody titers may remain positive even for years after resolution of the infection (Cutler *et al.* 1998a). However, the titer of specific antibodies has tendency to fall: a 25–50% drop in the IgG antibody titer in paired sera 6 months after treatment confirms eradication in most cases (Kosunen *et al.* 1992, Kato *et al.* 1999, Kolho *et al.* 2002, Bergey *et al.* 2003).

***Enzyme-linked immunosorbent assay (ELISA)*** is commonly used for detection of specific antibodies in the serum, as it is easy to perform, inexpensive, widely available, and suitable also for large-scale screening (Cutler 1998b). The meta-analysis of 21 studies with commercially available ELISA kits reported overall sensitivity of 85% and specificity of 79% (Loy *et al.* 1996). The performance of different assays may vary between patient populations from different geographic regions and ethnic backgrounds due to the differences between antigenic properties of bacterial strains colonizing patients (Bodhidatta *et al.* 1993, Hoang *et al.* 2004). Therefore, the test should be locally validated. In young children, the sensitivity of ELISA is low when the cut-off values validated for adults are used (Raymond *et al.* 1996, Khanna 1998, de Oliveira *et al.* 1999b, Kindermann *et al.* 2001).

***Immunoblot*** is more costly and time-consuming than ELISA, but permits a detailed analysis of antibody reactivity to the different *H. pylori* proteins. It has been a useful complement to serology in children, particularly in cases with doubtful ELISA results (Raymond *et al.* 2000, Rocha *et al.* 2000).

Several near-patient or office-based tests on the whole blood have been developed, they are quick and easy to perform, however, the diagnostic accuracy of these tests is inadequate for recommending them for widespread usage (Roberts *et al.* 2000, Vaira & Vakil 2001). Tests on the saliva and urine are attractive because samples can be easily obtained. Salivary tests have shown

inconsistent results with less than optimum sensitivity and specificity, urine antibody tests have demonstrated accuracy comparable to that of serum-based ELISA (Kabir 2003).

### 4.4.3. Urea breath test

Urea breath test (UBT) is based on the capacity of *H. pylori* to secrete the enzyme urease, which hydrolyses urea to ammonia and carbon dioxide. The test substance used is urea, labelled with a carbon isotope, either with $^{14}C$ (a radioactive isotope) or $^{13}C$ (a stable isotope). The $^{13}C$-UBT is most frequently employed for its safety; however, the cost of $^{13}C$-UBT is higher than of $^{14}C$-UBT. In non-infected individuals ingested urea leaves the stomach unchanged, while in infected subjects urea is split into ammonia and $CO_2$, which can be detected in the expired air. Labelled urea is usually given to the patient with a test meal to delay gastric emptying and to increase the time of contact with the mucosa.

The UBT detects current *H. pylori* infection with a sensitivity and specificity of >95% (Bazzoli *et al.* 2000, Vaira & Vakil 2001). The test is indicated for initial diagnosis of the infection and for follow-up of eradication therapy; in the latter case, the UBT should not be performed before an interval of four weeks has elapsed (Malfertheiner *et al.* 2002). In some studies a lower specificity of UBT has been found in children younger than 2–6 years of age (Kindermann *et al.* 2000, Imrie 2001), in other studies specificity has been high even in children less than 6 years of age (de Carvalho Costa Cardinali *et al.* 2003, Machado *et al.* 2004). False-negative results may occur during treatment with proton pump inhibitors (Laine *et al.* 1998).

### 4.4.4. Tests based on detection of bacterial antigens or bacterial DNA in faeces

Identification of bacterial antigens in stool using enzyme immunoassays has emerged in recent years, and tests based on polyclonal antibodies (Vaira *et al.* 1999) and monoclonal antibodies (Makristathis *et al.* 2000, Agha-Amiri *et al.* 2001) have been developed. A systematic review of 89 studies including 10,585 patients concluded that stool antigen test is an accurate method for diagnosing *H. pylori* infection: the mean sensitivity and specificity were 91% and 93%, respectively (Gisbert & Pajares 2004a). Overall results revealed that tests based on monoclonal antibodies are slightly more sensitive and specific and have greater reproducibility than tests based on polyclonal antibodies (Gisbert & Pajares 2004a).

PCR-based methods of detecting *H. pylori* DNA in stool samples have also been developed (Li *et al.* 1996, Casswall *et al.* 1999, MacKay *et al.* 2003). They

allow genotyping of strains, detection of virulence factors and testing of antibiotic susceptibility (Fontana *et al.* 2003).

## 4.5. Treatment of *H. pylori* infection

The goal of treatment of *H. pylori* infection is complete eradication of the organism. Eradication is currently defined as absence of *H. pylori* for a minimum of 4 weeks after treatment, as confirmed by biopsy-based test, UBT or stool antigen test (Malfertheiner *et al.* 2002). Clinically relevant eradication regimens must have cure rates of at least 80%, according to intention-to-treat analysis, without major side effects and with minimal induction of bacterial resistance (Suerbaum & Michetti 2002).

### 4.5.1. Indications for treatment

Eradication of *H. pylori* infection is an effective way to cure *H. pylori*-associated peptic ulcer disease, as it facilitates ulcer healing, prevents recurrences in vast majority of patients (Ford *et al.* 2004), and significantly reduces the risk of complications (Gisbert *et al.* 2004b).

Eradication of *H. pylori* infection leads to resolving of gastric inflammation both in adults and in children (Oderda *et al.* 1992, Hojo *et al.* 2002), and it may lead to reversion of existing atrophy in the gastric mucosa (Correa *et al.* 2000, Hojo *et al.* 2002, Kokkola *et al.* 2002). Eradication of *H. pylori* induces remission of gastric MALT lymphoma (Wotherspoon *et al.* 1993) and would likely prevent development of gastric cancers if intervention comes early enough in the sequence of carcinogenesis (Uemura *et al.* 2001, Wong *et al.* 2004).

Whether the eradication of *H. pylori* results in improvement of symptoms in patients with non-ulcer dyspepsia or not is still debatable. Meta-analyses of randomized controlled trials have yielded equivocal results: one showed no improvement of symptoms with *H. pylori* eradication (Laine *et al.* 2001), while another revealed a statistically significant but small benefit (Moayyedi *et al.* 2003). At the best, one case of non-ulcer dyspepsia is being cured for every 10 persons whom the infection was eradicated (Moayyedi *et al.* 2003); at the worst, eradication induces exacerbation of gastro-oesophageal reflux disease (Wu *et al.* 2004).

A number of guidelines on management of *H. pylori* infection have been compiled, based on published clinical evidence and expert opinion, as the Maastricht 2–2000 Consensus Report (Malfertheiner *et al.* 2002) and guidelines the from European Society of Primary Care Gastroenterology (ESPCG) (Rubin *et al.* 1999). In Estonia, guidelines on management of *H. pylori* infection were

developed in 1998 in collaboration of the Estonian Society of Family Doctors and the Estonian Gastroenterologists' Society (Labotkin *et al.* 1999).

Some consensus statements specifically address to the management of *H. pylori* infection in children, as the statement of the Canadian *Helicobacter* Consensus Conference on the approach to *Helicobacter pylori* infection in children and adolescents (Sherman *et al.* 1999), a consensus statement of European Pediatric Task Force on *Helicobacter pylori* (Drumm *et al.* 2000), and a medical position statement of the North American Society for Pediatric Gastroenterology and Nutrition (NASPGN) (Gold *et al.* 2000).

All guidelines and consensus statements recommend testing for *H. pylori* infection only when subsequent treatment of *H. pylori* infection is planned, and recommend eradication of *H. pylori* in patients with peptic ulcer disease. Other recommendations vary somewhat between different guidelines according to the population and setting applied (Table 4).

All the above pediatric consensus statements agree that children should be investigated for *H. pylori* infection only when they present with symptoms and signs suggestive of organic disease, such as peptic ulcer, and that optimal approach to investigation for *H. pylori* infection is upper gastrointestinal endoscopy with multiple biopsies. "Test-and-treat" strategy, *i.e.* verifying presence of *H. pylori* infection by UBT or stool antigen test and prescribing anti-*H. pylori* treatment without concomitant gastroscopy, is not recommended in children. There is some disagreement regarding whether *H. pylori*–positive children with abdominal complaints but without peptic ulcer should receive eradication treatment. The NASPGN guidelines state that there is no compelling evidence to recommend eradication treatment (Gold *et al.* 2000), whereas the proceedings of the European and Canadian consensus conferences state that if symptoms are severe enough to justify endoscopic evaluation, and if *H. pylori* infection is found, then the treatment is indicated (Sherman *et al.* 1999, Drumm *et al.* 2000).

**Table 4.** Recommendations for treatment of *H. pylori* infection according to different guidelines and consensus statements

| | Maastricht 2–2000 Consensus Report | Guidelines from the ESPCG | Statement of European Pediatric Task Force on *H. pylori* | Statement of the NASPGN |
|---|---|---|---|---|
| peptic ulcer disease (present or past) | strongly recommended | consider therapy | recommended | recommended |
| MALT lymphoma | strongly recommended | NA | NA | recommended |
| chronic gastritis | strongly recommended in atrophic gastritis | NA | recommended if *H. pylori* infection was diagnosed on gastroscopy | recommended, if atrophic gastritis with intestinal metaplasia |
| post-gastric cancer resection | strongly recommended | NA | NA | NA |
| family members of *H. pylori*-positive patient | strongly recommended for first-degree relatives of gastric cancer patients | not recom-mended | NA | not recom-mended |
| patient's wish | strongly recommended (after consultation with physician) | NA | NA | NA |
| non-ulcer dyspepsia, recurrent abdominal pain | recommended | not recom-mended | recommended if *H. pylori* infection was diagnosed as a result of gastroscopy | no compelling evidence to recommend |
| GERD | recommended | NA | NA | NA |
| regular NSAIDs usage | recommended | NA | NA | NA |
| possible extragastric manifestations of *H. pylori* infection | NA | NA | NA | not recommended in case of idiopathic short stature |
| asymptomatic individuals | NA | NA | NA | screening is not recommended |

NA – not addressed

### 4.5.2. Combined antibacterial regimens against *H. pylori* infection

*H. pylori* is susceptible to many antibiotics *in vitro*, *e.g.* penicillin, ampicillin, cefotaxime, erythromycin and tetracycline (Lambert *et al.* 1986). *In vivo*, however, the effectiveness of a treatment with a single agent is very low, less than 15% (Laheij *et al.* 1999). For a successful treatment, combined antibacterial therapy is needed, and because low acidity influences the effectiveness of some antimicrobial agents, combinations with proton-pump inhibitors or ranitidine are often used.

A large number of different medicaments and their combinations have been evaluated to find optimal therapeutic regimen(s), and optimizing of treatment is an ongoing challenge. Laheij *et al.* (1999) reviewed studies performed up to 1998 and identified 132 different medication combinations used. Oderda *et al.* (2000) reviewed studies done on pediatric population until 1999, and found that a total of 22 different treatment combinations were used. The most effective treatment combinations in adults comprise with three medicaments: a so-called proton pump inhibitor based triple therapy which consists of a proton pump inhibitor together with two antibiotics, usually clarithromycin with either amoxicillin or metronidazole, or so-called classic triple therapy consisting of a bismuth compound together with metronidazole and tetracycline or amoxicillin (Lahej *et al.* 1999). The cure rates of different triple regimens, analyzed on intention-to-treat basis, are roughly 75–85% (Lahej *et al.* 1999, Ulmer *et al.* 2003).

The recommended first-line therapy for adults according to the Maastricht 2–2000 Consensus Report is a proton pump inhibitor (alternatively ranitidine bismuth citrate) combined with clarithromycin and amoxicillin or metronidazole (Malfertheiner *et al.* 2002). The recommended duration of the treatment ranges from 7 to 14 days (Howden & Hunt 1998, Malfertheiner *et al.* 2002).

In children, few randomized double-blinded trials have been performed on eradication of *H. pylori* infection (Gottrand *et al.* 2001, Oderda *et al.* 2004, Tindberg *et al.* 2004, Ashorn *et al.* 2004), and the best treatment strategies have to be identified. Existing data indicate that dual therapies might be as effective as triple therapies (Oderda *et al.* 2000, Tindberg *et al.* 2004, Oderda *et al.* 2004). The treatment recommendations given in consensus statements are based on adult data: statement of the Canadian *Helicobacter* Consensus Conference suggests a 2-week course of a triple regimen with a proton pump inhibitor in combination with two antibiotics (Sherman *et al.* 1999). The NASPGN statement suggests a triple or quadruple therapy given for 1 or 2 weeks (Gold *et al.* 2000), whereas the European Pediatric Task Force on *Helicobacter pylori* has not given any specific recommendations regarding optimal treatment due to the insufficient data in relation to this issue (Drumm *et al.* 2000).

### 4.5.3. Factors influencing treatment results

None of the regimens cure *H. pylori* infection in 100% of the patients; a number of factors, as bacterial resistance to antibiotics, patient compliance, high bacterial load, low gastric pH and others may affect outcome of eradication therapy (Mégraud & Lamouliatte 2003).

The most important reason for treatment failure appears to be drug resistance, especially clarithromycin-resistance, which leads to about 70% decrease in efficacy of the clarithromycin-containing regimen (Mégraud 2004), whereas the impact of metronidazole resistance on clinical outcome is relatively modest, leading to about 25% decrease in efficacy (Mégraud 2004). The controversy can be attributed to the methodological problems in determination of metronidazole resistance of *H. pylori* (Mégraud *et al.* 1999, Lõivukene *et al.* 2000). The pattern of resistance to clarithromycin and metronidazole varies within wide limits in different regions (Glupczynski *et al.* 2001, Boyanova *et al.* 2002, Wheeldon *et al.* 2004). In Estonia, 46% of the *H. pylori* isolates collected in 1995–2000 were resistant to metronidazole and 3% were resistant to clarithromycin (Lõivukene *et al.* 2002). The resistance of *H. pylori* to amoxicillin and tetracycline is low, less than 1% of isolates worldwide (Mégraud *et al.* 1999, Glupczynski *et al.* 2001, Boyanova *et al.* 2002, Lõivukene *et al.* 2002**,** Duck *et al.* 2004). The primary or acquired resistance of *H. pylori* to bismuth salts has never been reported (Lambert & Midolo 1997).

The other important determinant of successful eradication therapy is patient's compliance with the prescribed regimen (Graham *et al.* 1992, Wermeille *et al.* 2002). The common reason of non-compliance is occurrence of side effects (Wermeille *et al.* 2002), while convenience also plays a role: patients receiving a 2–3-times-a-day regimen were significantly more compliant than those receiving a 4-times-a-day regimen (Fennerty *et al.* 1999).

Eradication treatment appears to be more effective in patients with peptic ulcer disease than without it (Broutet *et al.* 2003, Oderda 2003), irrespective of patient compliance (Broutet *et al.* 2003).

### 4.5.4. Alternatives for treatment of *H. pylori* infection

The suboptimal success rate of current antibacterial regimens and emerging antibiotic resistance has lead to the search of new modalities to treat *H. pylori* infection. Investigation of the possible role of probiotics in the adjunctive treatment and prophylaxis of *H. pylori* infection has yielded encouraging results (Hamilton-Miller 2003). The effects of medicinal plants on *H. pylori* have been studied as well (Annuk *et al.* 1999). Bovine lactoferrin in combination with antibiotic therapy has improved the *H. pylori* eradication rate (Di Mario *et al.* 2003).

One of alternative approaches is passive immunization with orally administered antibodies. One of the best accessible sources of immunoglobulins is bovine colostrum, the milk secreted by cows during the first four days after parturition. The bovine colostrum contains approximately 60 g immunoglobulins per liter (range 30–200 g/l); majority of it, over 75% comprises $IgG_1$ (Korhonen *et al.* 2000a). The concentration of specific antibodies against pathogens in the colostrum can be increased by immunizing cows with these pathogens or their antigens (Korhonen *et al.* 1995, Casswall *et al.* 2002). Such colostrum is referred to as immune colostrum or hyperimmune colostrum. Orally administered bovine immune colostrum (BIC) has been shown to provide effective protection against various gastrointestinal infections such as rotaviral infection, shigellosis, and infection with enterotoxigenic *E. coli* (Korhonen *et al.* 2000b, Kelly 2003). Successful therapeutic interventions have been reported in childhood rotaviral infections and cryptosporidial infections in immunocompromized patients (Korhonen *et al.* 2000b). *In vitro*, BIC exhibits high bactericidal activity against *H. pylori* (Korhonen *et al.* 1995, Marnila *et al.* 2003) and inhibits *H. pylori* adherence to the gastric mucosa in a dose-dependent manner (Casswall *et al.* 2002).

Promising results have been obtained in animal experiments: mice given monoclonal IgA anti-*H. felis* antibodies at the time of initial challenge were protected from infection (Czinn *et al.* 1993). Marnila *et al.* (2003) demonstrated that the BIC derived from cows immunized with *H. felis* but not control colostrum protected some mice against infection: after an experimental challenge, 10 out of 17 mice in immune preparation group remained *H. felis* negative *versus* none out of 18 mice in control group ($p<0.005$). Casswall *et al.* (2002) reported eradication of *H. pylori* infection in mice receiving BIC. The question whether bovine immune colostrum has some therapeutic potential for human *H. pylori* infection was addressed in the present study.

### 4.5.5. Recurrence of *H. pylori* infection after eradication

Recurrence refers to a situation where tests for *H. pylori* infection, which were negative at four weeks after cessation of treatment, become positive at a later stage (Xia *et al.* 1997).

Recurrence is the result of either reinfection or recrudescence. Recrudescence is a situation where the pre-treatment strain of *H. pylori*, which was suppressed by treatment and was undetectable one month after treatment, becomes detectable at a later stage, usually within first three months after treatment. Reinfection is acquisition of a new infection, either a new strain of *H. pylori* or a genetically identical strain, after the original strain of *H. pylori* has been completely eradicated (van der Ende *et al.* 1997, Xia *et al.* 1997, Hildebrand *et al.* 2001).

Due to the high grade of genetic diversity of *H. pylori* strains among unrelated patients (Go *et al.* 1996), distinguishing between recrudescence and true reinfection by comparing pre- and post-treatment isolates by genotyping methods is suggested (Xia *et al.* 1997). However, the results of genetic analysis of the strains should be interpreted with caution, since an individual patient may be colonized with multiple strains (van der Ende *et al.* 1996, Leal-Herrera *et al.* 2003), also there may occur reinfection by a strain identical to the one present prior to treatment.

The recurrence rate during the first 6–12 months is inversely associated with the efficacy of the original course of eradication therapy (Xia *et al.* 1995, Bell & Powell 1996, Gisbert *et al.* 1998, Seo *et al.* 2002b). This suggests that in cases of low effectivity treatment, most recurrences that occur shortly after treatment are actually recrudescences.

The risk of reinfection appears to be different in different regions of the world (Table 5). In industrialized countries, reinfection is a rare event: the reported recurrence rates in adults are generally lower than 2% per patient-year. The lowest recurrence rate, virtually no recurrences during 2 years after treatment has been reported in a study conducted in Germany (Knippig *et al.* 2002). In developing countries, the reported recurrence rates are variable, being generally higher than 5% per patient-year. The highest reinfection rates, >15% per patient-year, have been reported from Peru and Bangladesh (Hildebrand *et al.* 2001, Soto *et al*. 2003). A recurrence rate as low as 1.1% per patient-year was found in a study conducted in China (Mitchell *et al.* 1998).

Only a few studies have addressed post-treatment recurrence rates of *H. pylori* infection in children, and they have been conducted mostly in areas where the current incidence of *H. pylori* infection among children is low. It seems that the risk for recurrence is slightly in children higher than in adults in the same region, ranging between 2.0–5.8% per patient-year (Table 6).

Prevalence of the infection among family members seems not to predict recurrence rate. In Germany, there were no recurrences detected in 96 successfully treated adult patients during a 2–year follow-up in spite of the fact that 56% of the spouses were infected (Knippig *et al.* 2002). The reinfection was an unlikely event also among Irish children (reinfection rate 2.0% per patient-year), even though 66% of the siblings, 78% of the mothers and 65% of the fathers in this study were infected, and having infected parents and siblings was not an independent risk factor for recurrences (Rowland *et al.* 1999). Farrell *et al.* (2004) conducted a prospective randomized study to investigate whether eradication of *H. pylori* in the entire family reduces the risk of childhood reinfection, and found no significant effect. These data suggest that *H. pylori* eradication therapy should not prescribed for infected spouses or siblings with the aim to prevent reinfection of the patient.

**Table 5.** Recurrence rates of *H. pylori* infection after eradication in adults

| Reference | Country | Duration of follow-up | Recurrence rate (% per patient-year) |
|---|---|---|---|
| Knippig *et al.* 2002 | Germany | 2 years | 0% |
| Borody *et. al.* 1994 | Australia | 4–8 years | 0.4%[a] |
| Miehlke *et al.* 1996 | Germany | mean 25 months (up to 5 years) | 0.8% (during the first 2 years)<br>0.3% (during the 3rd and 4th years) |
| Mitchell *et al.* 1998 | China | 2 years | 1.1% |
| van der Hulst *et al.* 1997 | Netherlands | mean 3.5 years (range 1.0–9.2 years) | 1.2% |
| Adachi *et al.* 2002 | Japan | 2 years | 1.2% (during the 1st year)<br>1.5% (during the 2nd year) |
| Bell *et al.* 1996 | UK | up to 8 years | 1.3%[b] |
| Figueroa *et al.* 1996 | Chile | 12 months | 4.2% |
| Gisbert *et al.* 1998 | Spain | up to 2 years | 4.2% |
| Khor *et al.* 2000 | Singapore | 9–36 months | 5.4% |
| Gisbert *et al.* 2002 | Spain | 12 months | 6.8% |
| Archimandritis *et al.* 1999 | Greece | mean 25 months (up to 6 years) | 9.7% (during the 1st year)<br>2.3% (after the 1st year) |
| Della Libera *et al.* 2001 | Brazil | 12 months | 7.6% |
| Rollan *et al.* 2000 | Chile | 3 years | 8% (during the 1st year);<br>cumulative recurrence rate 13% |
| Leal-Herrera *et al.* 2003 | Mexico | 24 months | 15.6% (during the 1st year)<br>6.3% (during the 2nd year) |
| Hildebrand *et al.* 2001 | Bangladesh | up to 22 months | 18.0%[c]<br>15.4% (during the 1st year)<br>23.4% (during the 2nd year |
| Soto *et al.* 2003 | Peru | 18 months | cumulative recurrence rate 30.3%[d] |

[a] for the period beyond one year after treatment
[b] for the period beyond 6 months after treatment
[c] for the period beyond 3 months after treatment, pre-and post-treatment strains of *H. pylori* were different in all cases
[d] pre- and post-treatment strains of *H. pylori* were different in 79% of cases

**Table 6.** Recurrence rates of *H. pylori* infection after eradication in children

| Reference | Country | Mean age of patients at the beginning of the study (range) | Mean duration of follow-up (range) | Recurrence rate (% per patient-year) |
|---|---|---|---|---|
| Kato *et al.* 1998 | Japan | 12 (5–16) | 22 months (12–34 months) | 2.4% |
| Rowland *et al.* 1999 | Ireland | 11.5 (1–17) | 24 months (6–62 months) | 5.8%, 2.0% for those aged >5 years |
| Feydt-Schmidt *et al.* 2002 | Germany | (1–18) | 15.5 months | 2.3% |
| Farrell *et al.* 2004 | Northern Ireland, UK | 9.5 | 62.2 months | 2.4% |

# 5. AIMS OF THE STUDY

1. To determine the prevalence of *H. pylori* infection among children aged 9–15 years and living in Southern Estonia (paper I).
2. To evaluate the dynamics of the prevalence of *H. pylori* infection among children in Estonia during an 11-year period of profound socioeconomic changes (1991–2002) (papers II, V).
3. To determine long-term recurrence rate after combined antibacterial treatment of *H. pylori* infection, and to evaluate the risk factors of recurrent infection in children and adolescents in Estonia (paper III).
4. To evaluate the effect of administration of bovine immune colostrum, an alternative to the antibiotic treatment, on *H. pylori* infection and on chronic gastritis (paper IV).

# 6. SUBJECTS AND METHODS

Overview of the study designs, subjects and methods for diagnosing *H. pylori* infection is presented in Table 7.

**Table 7.** Study designs, subjects, and methods for diagnosing *H. pylori* infection

| Aim of the study | Study design | Study subjects | Methods of detection of *H. pylori* infection | Paper |
|---|---|---|---|---|
| Determination of the prevalence of *H. pylori* infection | a cross-sectional study | randomly selected children aged 9, 12, 15 years, living in Southern Estonia, n=421 | ELISA | I |
| Evaluation of the dynamics of the prevalence of *H. pylori* infection among children in Estonia 1991–2002 | comparison of cross-sectional surveys | consecutively hospitalized children during predefined time periods in 1991 (n=425) and in 2002 (n=296) | ELISA and immunoblot (in cases of borderline ELISA results) | II, V |
| Determination of long-term recurrence rate after combined antibacterial treatment of *H. pylori* infection | a prospective follow-up study | consecutive patients with RAP and *H. pylori* infection, who completed a treatment course against *H. pylori* infection 1993–1995, n=27 (at the beginning of the study), n=23 (at the end of the study) | histology and rapid urease test before treatment and 4–6 weeks after treatment, $^{13}$C-UBT in 1997 and in 2002 | III |
| Evaluation of the effect of administration of bovine immune colostrum on *H. pylori* infection and on chronic gastritis | a non-randomized non-controlled trial (pilot study) | children with recurrent abdominal pain and *H. pylori* positive chronic gastritis, n=20 | histology and rapid urease test before treatment and within 2 weeks after treatment | IV |

## 6.1. Study designs, subjects and settings

### 6.1.1. Determination of the prevalence of *H. pylori* infection among children living in Southern Estonia

In order to determine the prevalence of *H. pylori* infection among children, a community-based cross-sectional study was carried out in five Southern Estonian counties (Jõgeva, Põlva, Tartu, Valga, Võru) (paper I). The subjects were 9-, 12- and 15-year-old schoolchildren from 20 secondary schools. The schools were selected assuming that a similar number of study subjects are recruited from each county. Within each school, every third 9-year-old child (age range 8.5–9.6 years), 12-year-old child (age range 11.5–12.6 years) and 15-year-old child (age range 14.5–15.6 years) from the school roll was asked to participate in the study. The sera were collected from autumn 1993 to spring 1996. The same sera were also used for assessing the cardiovascular risk profile in Estonian children (Grünberg & Thetloff 1998). The total number of eligible children was 1377. In 1074 (78%) cases the child and the parent gave their written consent to participate. Of these children 56 were excluded due to following reasons: absence from school on the day of the blood sampling (n=16), reluctance to undergo blood sampling despite prior agreement (n=13), inadequately completed questionnaires (n=20), presence of a chronic disease, as diabetes, asthma, *etc.* (n=7). Thus, sera were collected from 1018 children, which accounted for almost 10% of the children of this age living in the given region. Of these subjects, sera from 421 (41%) were available for the *H. pylori* serosurvey, in the remaining cases the sera had been used up for other analyses. The distribution of the children by age, gender and place of residence in present study is given in the Table 8.

**Table 8.** Distribution of the studied children (n=421) by age, gender and place of residence

| Age | Gender | | Place of residence | | Total |
|---|---|---|---|---|---|
| | Girls | Boys | Urban | Rural | |
| 9 years | 47 (50%) | 47 (50%) | 39 (42%) | 55 (58%) | 94 (22%) |
| 12 years | 78 (53%) | 69 (47%) | 78 (53%) | 69 (47%) | 147 (35%) |
| 15 years | 106 (59%) | 74 (41%) | 123 (69%) | 57 (31%) | 180 (43%) |
| Total | 231 (55%) | 190 (45%) | 240 (57%) | 181 (43%) | 421 (100%) |

### 6.1.2. Evaluation of the dynamics of the prevalence of *H. pylori* infection in children in Estonia 1991–2002

In order to evaluate the dynamics of the prevalence of *H. pylori* infection in children in Estonia, two cross-sectional serosurveys were carried out in 1991 and 2002 on independent study populations selected according to the same inclusion criteria, and the results of the serosurveys were compared (papers II, V). The inclusion criteria were: all subjects were hospitalized in the Children's Clinic of Tartu University Clinics during predefined time periods in 1991 and in 2002, the age of the subjects was 6 months to 15 years, and at least one serum sample was obtained for various clinical analyses during hospitalization with no less than 0.1 ml of serum left over from the analyses. Children less than 6 months of age were excluded because they may show false positive results due to persistence of transplacentally acquired maternal IgG antibodies (Blecker *et al.* 1994, Ashorn *et al.* 1996).

Group 1991 consisted of 425 patients hospitalized between 1 January and 31 May 1991, it accounted for 24.2% of the 1754 children hospitalized in the Children's Clinic of Tartu University Clinics during the given time period. Their serum samples had been initially obtained for a screening of celiac disease (Uibo & Maaroos 1993). Originally, 700 serum samples were collected from 666 patients, the sera of 241 of these patients were not analyzed in this study for the following reasons: in 204 cases the sera were not stored long-term because there was too little serum; in 28 cases, coding did not allow identification of the patient data, and in nine cases the patients were younger than 6 months or older than 15 years.

Group 2002 consisted of 296 patients hospitalized between 1 February and 31 May 2002, it accounted for 19.5% of the 1518 children hospitalized in the Children's Clinic of Tartu University Clinics during the given time period. The sera were collected for this particular study.

There were no statistically significant differences between the two groups in gender and place of residence, but there were differences in the distribution by age and by some of the diagnoses (Table 9). The diagnoses whose distribution revealed no statistically significant differences were summarized and presented under the category "others".

The study base, Children's Clinic of Tartu University Clinics, is one of the two regional pediatric hospitals in Estonia, serving a study and research base for the Faculty of Medicine of the University of Tartu. Majority of the patients live in the city and county of Tartu and in four adjacent counties (Jõgeva, Põlva, Valga, Võru), however, in the case of diagnostic and treatment difficulties, patients from all over Estonia are admitted in this hospital.

**Table 9.** Characteristics of the patients by the year of enrolment

| | No. (%) of children | | *p*-value |
|---|---|---|---|
| | Group 1991 n=425 | Group 2002 n=296 | |
| Age (years) | | | <0.0001 |
| 0.5–5 | 186 (44) | 180 (61) | |
| 6–10 | 133 (31) | 51 (17) | |
| 11–15 | 106 (25) | 65 (22) | |
| Gender | | | NS |
| boys | 228 (54) | 148 (50) | |
| girls | 197 (46) | 148 (50) | |
| Region of residence | | | NS |
| city and county of Tartu | 189 (44) | 149 (50) | |
| adjacent counties | 101 (24) | 65 (22) | |
| more distant counties | 135 (32) | 82 (21) | |
| Place of residence | | | NS |
| urban | 247 (58) | 182 (61) | |
| rural | 178 (42) | 114 (39) | |
| Diagnosis | | | |
| recurrent abdominal pain (306.4[a], 536.9[a], 575.8[a], F45.3[b]) | 45 (11) | 1 (<1) | <0.0001 |
| acute intestinal infections (003–009[a], A03–A09[b]) | 13 (3) | 52 (18) | <0.0001 |
| epilepsy (345[a], G40[b]) | 10 (2) | 22 (7) | <0.005 |
| other | 357 (84) | 221 (75) | <0.005 |

[a] disease codes according to the ICD-9, Group 1991
[b] disease codes according to the ICD-10, Group 2002

### 6.1.3. Determination of long-term recurrence rate after treatment of *H. pylori* infection

In order to determine the long-term recurrence rate after treatment of *H. pylori* infection in children and adolescents and to evaluate the risk factors of recurrent infection, a prospective follow-up study was carried out (paper III).

The patients were selected according to the following inclusion criteria: investigated by upper gastrointestinal endoscopy at the Children's Clinic of Tartu University Clinics due to abdominal complaints in 1993–1995, appeared to be *H. pylori* positive at histological examination and rapid urease test, and completed a treatment course against *H. pylori* infection.

All eligible patients were asked to attend a post-treatment follow-up endoscopy 4–6 weeks after completion of therapy (1st follow-up visit), and invited for follow-up check-up by $^{13}$C-UBT in 1997 (2nd follow-up visit) and 2002 (3rd

follow-up visit). During all visits, the patients were interviewed and a questionnaire was filled in.

In 1993–94, treatment of *H. pylori* infection was prescribed to four *H. pylori* positive children with duodenal ulcer. All of them completed the treatment with no recorded side effects. In 1995, the treatment was prescribed to all 30 *H. pylori* positive children. Altogether 56 patients were studied by upper gastrointestinal endoscopy that year; 30 (54%) of them were *H. pylori* positive, and four had duodenal ulcers. Twenty-three children completed the treatment; 4 were non-compliant, the treatment was interrupted in 2 children because of side effects (one due to allergic reaction and the another due to severe nausea) and in 1 patient the treatment was terminated due to concomitant porphyria.

Thus, a total of 27 patients, 4 treated in 1993–94 and 23 treated in 1995, were invited for follow-up visits. The mean age of these patients at the time of the treatment was 13.3±2.3 years (range 7–17 years); 15 were boys, and 12 were girls; 14 of them lived in towns and 13 lived in rural areas.

### 6.1.4. Evaluation of the effect of administration of bovine immune colostrum on *H. pylori* infection and on the chronic gastritis

In order to evaluate the effect of immune bovine colostrum on *H. pylori* infection and on the chronic gastritis, a pilot trial was carried out (paper IV). The study group consisted of 20 children enrolled according to the following inclusion criteria: they had been referred to the upper gastrointestinal endoscopic examination to Children's Hospital of the University of Tartu, had a history of recurrent abdominal pain, had no peptic ulcer or mucosal erosions at endoscopy, and were *H. pylori* positive according to the rapid urease test performed on the antrum mucosa. The patients and their parents gave written informed consent to participate. None of the patients had received treatment with antibiotics, $H_2$–antagonists or proton pump inhibitors within 4 weeks before the study. The mean age of the patients was 12.4 years (range 10–17 years), eight of them were boys and 12 were girls. The study participants were selected out of 66 consecutive children studied by upper gastrointestinal endoscopy during a 12-month period in Nov 1993–Oct 1994, 36 of them were excluded due to the negative rapid urease test result, 3 due to duodenal ulcer and one to gastric mucosal erosions, 7 did not agree to participate.

Before the treatment and within 2 weeks after the discontinuation of the treatment, the patients and their parents were interviewed, and upper gastrointestinal endoscopy with obtaining biopsy samples from the antrum and from the corpus mucosa was performed. Both prior to and after treatment, two biopsies from the antrum and two from the corpus were examined histologically, and a rapid urease test was made using an antrum biopsy sample.

## 6.2. Methods of detection of *H. pylori* infection

### 6.2.1. Serological tests

Sera were tested for *H. pylori* IgG antibodies by using the in-house ELISA (papers I, II) and, in the case of borderline ELISA results, by the in-house immunoblot assay (paper II). The ELISA and immunoblot methodologies were developed at Lund University (Guruge *et al.* 1990, Nilsson *et al.* 1997). The same methodologies have been used for other seroepidemiological studies conducted in Estonia (Vorobjova *et al.* 1994, Lindkvist *et al.* 1999a). Acid-glycine-extracted cell surface proteins of the *H. pylori* strain NCTC 11637 were used as the antigen for both assays (Guruge *et al.* 1990). For paper II, each ELISA plate and each set of immunoblot strips contained serum samples from Group 1991 and Group 2002 proportionately to their overall amounts.

***ELISA*** was performed as described previously (Guruge *et al.* 1990). The ELISA results were expressed as the corrected mean absorbance values as the percentage of a reference standard (human gamma-globulin, Pharmacia & Upjohn, Stockholm, Sweden). The cut-off value for seropositivity was a relative antibody activity (RAA) of 25, derived from the mean value of RAA plus two standard deviations (Brown & Swanson Beck 1989) for 39 histologically *H. pylori*-negative children with median age of 11 years (range 10–16 years). The specificity and the sensitivity of the test were 100% and 77%, respectively, as determined with the sera of 39 children aged 4–15 years whose gastric biopsy samples were examined histologically for *H. pylori* infection (Maaroos *et al.* 1993). In paper II, all RAA values from 20 to 30 were considered borderline, and confirmative immunoblot analysis was carried out.

***Immunoblot assay.*** The immunoblot assay was performed as described previously (Nilsson *et al.* 1997). Sera staining with a high $M_r$ protein (120 kDa CagA) alone or with the 87, 33 and 30 kDa proteins were scored as *H. pylori* positive (Nilsson *et al.* 1997). Two investigators unaware of the ELISA results evaluated the results independently.

### 6.2.2. Histological examination of the gastric mucosa

Gastric mucosal samples were taken during upper gastrointestinal endoscopy using an Olympus GIF p12 gastroscope. Before the procedure, the pharynx was anaesthetized with 10% xylocaine. The biopsy specimens were taken with standard biopsy forceps both from the antrum mucosa within 2 cm proximal to the pylorus and from the corpus mucosa. 5 μm thick sections of formalin-fixed, paraffin-embedded biopsy specimens were stained with Giemsa stain for the

assessment of *H. pylori* colonization and with hematoxylin and eosin for the assessment of gastritis. Endoscopic and histological evaluations were performed according to the Sydney system (Tytgat 1991, Price 1991).

### 6.2.3. Rapid urease test

An antrum mucosa specimen was used for rapid urease test (Jatrox®-test, Röhm Pharma Gmbh). A fresh biopsy sample was placed into the test media. If *H. pylori* was present in the tissue, the color of the test medium changed from opalescent to red. Color changes were generally detectable within a few minutes, a final assessment of the color of the test medium was performed after 1 hour.

### 6.2.4. $^{13}$C-urea breath test

The $^{13}$C-UBT was performed, after fasting for at least 4h, by the Helicobacter Test *Hp*-PLUS® (Utandiningstester I Sverige AB), in accordance with the manufacturer's instructions. Breath samples were collected in duplicate before and 30 min after the ingestion of $^{13}$C-urea, and were analyzed mass-spectrometrically in Meilahti Hospital, Helsinki University, Finland. Compared with the baseline, the results were expressed as $\delta\ ^{13}CO_2$ per mil (‰). The $\delta\ ^{13}CO_2$ of 5‰ or higher was considered positive for *H. pylori*, and $\delta\ ^{13}CO_2$ of 3–5‰ was considered borderline.

## 6.3. Recording of the other study variables

In order to evaluate the association between *H. pylori* seropositivity and place of residence (paper I), the data were derived from questionnaires completed by the children at school**.**

For paper II, the patient data (age, gender, region of residence, place of residence and diagnosis) were retrieved from case histories. The results of the histological examination of the gastric biopsies were drawn from database of the *H. pylori* study group of the University of Tartu.

For papers III and IV, all patients were interviewed by the researcher or by a trained nurse before treatment and during all follow-up visits, and a questionnaire was filled in. Before treatment, demographic data (age, gender), place of residence, education of the parents, medications taken within preceding months, history of abdominal complaints, frequency and localization of abdominal pain and the fact if it had disturbed daily activities or occurred at night, as well as

occurrence and frequency of other gastrointestinal symptoms (vomiting, nausea, heartburn, diarrhea, constipation) and occurrence of other complaints were recorded. After treatment, the abdominal symptoms were graded as “increased”, “unchanged”, “decreased” or “completely disappeared”, in comparison with the pre-treatment situation, and the occurrence of all other symptoms were recorded.

For paper III, questions about place of residence, number of the persons living at home, number and age of siblings living at home, number of living rooms in the family dwelling, type of water supply, keeping of pets or farm animals and use of medications taken in the preceding year were asked at all follow-up visits.

## 6.4. Treatment

### 6.4.1. Combined antibacterial treatment (paper III)

All children, with the exception of three in 1993, received triple treatment with amoxicillin (50 mg/kg/day, max. 3 g/day), metronidazole (15 mg/kg/day, max. 900 mg/day), and colloidal bismuth subcitrate (8 mg/kg/day, max. 480 mg/day) for 2 weeks. Three children in 1993 received dual therapy with amoxicillin (750 mg three times daily) and metronidazole (300 mg three times daily) for 2 weeks. The children with duodenal ulcer received additionally ranitidine 2–4 mg/kg/day, max. 300 mg/day for 4–6 weeks.

For recording compliance, the patients were asked to daily complete a table of the medications used. A patient was considered compliant when at least 60% of the medicaments of triple or dual treatment were taken (Graham *et al.* 1992).

### 6.4.2. Bovine immune colostrum (paper IV)

***Preparation of the bovine immune colostrum.*** BIC was produced by Valio Research and Developmental Centre as described previously (Korhonen *et al.* 1995). Briefly, cows were immunized before calving with a whole-cell vaccine made from the *H. pylori* strain NCTC 11637. Colostrum was collected from the first six milkings *post partum*. The freeze-dried BIC contained 56% immunoglobulins with high titers (>10 000) of specific bactericidal antibodies against *H. pylori* (Korhonen *et al.* 1995).

***Treatment protocol.*** 6 g immune bovine colostrum powder was diluted in 100 ml of lactose free milk and administered *per os* twice a day during 21 days. No other medicaments were administered during the treatment period and until the end of the follow-up. The patients were asked to return the remaining BIC packages.

## 6.5. Statistical methods

To test the differences in the proportions, chi-square test or Fisher's exact test was used as appropriate. The unpaired t-test was employed to compare the mean±SD of the continuous variables. All *p*-values calculated were two-tailed, *p*-values higher than 0.05 were considered non-significant (NS).

Mantel-Haenszel statistics was used to calculate odds ratios (OR) with 95% CI for the association between *H. pylori* serostatus and place of residence (paper I).

Multiple logistic regression analysis was used to determine the adjusted ORs with the 95% confidence intervals (95% CI) for *H. pylori* seropositivity for Groups 1991 and 2002 (paper II). The controlling variables, evaluated in the multiple logistic model, were age, gender, region of residence, place of residence and diagnosis. The variables were categorized as in Table 9 except for age, which was analyzed as a continuous variable. For estimating the possible biases due to the significant differences in the distribution of some of the diagnoses and for assessing if the patient's region and place of residence made different contributions to *H. pylori* seropositivity over time, additional logistic regression analyses were carried out separately for Groups 1991 and 2002. The logistic models included the following controlling variables: age, region of residence, place of residence, acute intestinal infection (yes/no), epilepsy (yes/no), recurrent abdominal pain (yes/no), the last variable was included in the analysis of Group 1991 only. The age-standardized *H. pylori* seroprevalence rates were calculated by direct standardization using Group 1991 as the standard population.

The calculation of the post-treatment recurrence rates of *H. pylori* infection was based on the patient-year method (Armitage & Perry 1987). Follow-up time for each particular patient (up to the recurrence if it occurred) was calculated. *H. pylori* recurrence rate (% per patient-year) was calculated as follows: the number of persons with recurrent infection, divided by the sum of the follow-up times for both *H. pylori*-negative and recurrent persons, was multiplied by 100. For the period between the 2$^{nd}$ and 3$^{rd}$ follow-up visits, the sum of follow up times was calculated on the basis of the assumption that the recurrence cases were equally distributed throughout this follow-up period.

The chi-square tests and Fisher's exact tests were performed and the confidence intervals of the proportions were calculated by INSTAT, Mantel-Haenszel test was performed with the Exact version 2.0, multiple regression analysis and t-tests were performed with the SPSS version 10.0.

## 6.6. Ethics

The studies were approved by the Ethics Committee on Human Research of the University of Tartu (papers I, II, III) and by the Medical Faculty Ethics Committee of the University of Tartu (paper IV).

# 7. RESULTS

## 7.1. Prevalence of *H. pylori* infection among children aged 9–15 years and living in Southern Estonia (paper I)

In a total of 56% (95% CI 51–61%) of the studied children, 235 out of 421 had *H. pylori* IgG antibodies in the serum. *H. pylori* prevalence rates according to gender, age, and place of residence are presented in Table 10.

**Table 10.** *H. pylori* prevalence rates for children in Southern Estonia according to gender, age and place of residence

| | No of *H. pylori*-positive/ No of subjects tested | Seroprevalence rate | *p*-value |
|---|---|---|---|
| Gender | | | NS |
| girl | 127/231 | 55% | |
| boy | 108/190 | 57% | |
| Age (years) | | | NS |
| 9 | 46/94 | 49% | |
| 12 | 81/147 | 55% | |
| 15 | 108/180 | 60% | |
| Place of residence | | | *p*=0.001 |
| urban | 117/240 | 49% | |
| rural | 118/181 | 65% | |
| Total | 235/421 | 56% | |

There were no statistical differences between the prevalence rates for different ages. The infection was more prevalent in children living in rural areas: the odds of being seropositive were 1.96 (95% CI 1.32–2.92) for children living in rural areas compared with children living in urban areas.

## 7.2. Dynamics of the prevalence of *H. pylori* infection in children in Estonia 1991–2002 (papers II, V)

The unadjusted results of the serological tests for Group 1991 and Group 2002 are presented in Table 11.

**Table 11.** Unadjusted results of ELISA and immunoblot assays for Groups 1991 and 2002

| *H. pylori* serostatus | No of patients (%) | | *p*-value |
|---|---|---|---|
| | Group 1991 | Group 2002 | |
| ELISA | **(n=425)** | **(n=296)** | |
| positive (RAA >30) | 156 (37%) | 64 (22%) | <0.0001 |
| negative (RAA <20) | 218 (51%) | 192 (65%) | |
| borderline (RAA=20–30) | 51 (12%) | 40 (13%) | |
| Immunoblot (only borderline ELISA cases) | **(n=51)** | **(n=40)** | NS |
| positive | 23 | 11 | |
| negative | 25 | 28 | |
| equivocal | 3 | 1 | |
| Summary results of ELISA and immunoblot | **(n=425)** | **(n=296)** | <0.0001 |
| positive | 179 (42%) | 75 (25%) | |
| negative | 243 (57%) | 220 (74%) | |
| equivocal | 3 (<1%) | 1 (<1%) | |

The results of the serological tests were compared with the results of the histological examinations of the gastric mucosa, which were available for 16 patients from Group 1991 but for none from Group 2002. In 8 cases both serological and histological examinations yielded positive results, while in 6 cases both results were negative. Discordant results were found in two cases: in one case ELISA was negative and histology positive, and in the other case *vice versa.* The borderline ELISA results were compared to the results of immunoblot: in 25 out of 34 (74%) immunoblot-positive cases, the RAA value was 25 and higher, and in 38 out of 53 (72%) immunoblot-negative cases the RAA value was less than 25. No statistically significant differences were found between Group 1991 and Group 2002 in the mean RAA values of the *H. pylori* positive sera, in the distribution of borderline ELISA results by age groups and in the concordance of ELISA and immunoblot results (Table 12).

**Table 12.** The results of the serological tests for Group 1991 and Group 2002

| | Group 1991 | Group 2002 | *p*-value |
|---|---|---|---|
| Mean RAA value (ELISA) | | | |
| *H. pylori*-positive sera | 60.7±25.7 | 59.2±30.6 | NS |
| *H. pylori*-negative sera | 7.4±10.8 | 10.5±7.2 | 0.002 |
| Distribution of the borderline ELISA results according to age | | | |
| 0–5 years | 29 | 26 | NS |
| 6–10 years | 11 | 6 | |
| 11–15 years | 11 | 8 | |
| Concordance between borderline ELISA and immunoblot results | | | |
| concordant | 33 | 30 | NS |
| discordant | 18 | 10 | |

The cases of the equivocal serological tests results were excluded from subsequent statistical analysis. Thus altogether 422 cases from Group 1991 and 295 cases from Group 2002 were analyzed.

According to multiple logistic regression analysis, the only two variables that were independently associated with *H. pylori* serostatus were age and year of enrolment: the adjusted odds of being *H. pylori* seropositive were 1.92 (95% CI 1.33–2.76) times higher for the children enrolled in 1991 compared with the children enrolled in 2002 (Table 13).

When the groups were analyzed by multiple logistic regression analysis separately, *H. pylori* serostatus was independently associated only with age: OR corresponding to the one-year age difference was 1.15 (1.09 – 1.21) for Group 1991 and 1.19 (1.12 – 1.28) for Group 2002; no association was found between *H. pylori* seropositivity and such variables as patient's place and region of residence as well as presence of acute intestinal infection or epilepsy. As there was only one patient with recurrent abdominal pain in Group 2002, the possible association of this diagnosis with *H. pylori* serostatus was assessed only for Group 1991, while no significant association was found: age-adjusted OR for seropositivity was 1.23 (95% CI 0.63 – 2.41) for the children with recurrent abdominal pain in comparison with the children without it ($p>0.05$).

The age-standardized *H. pylori* seroprevalence rate was 42.2% (95% CI 37.4–47.0%) in Group 1991 and 28.1% (95% CI 23.1–33.6%) in Group 2002 ($p=0.0002$). Stratified analysis by the age groups revealed that a statistically significant decrease in seroprevalence over the 11-year period occurred for the two younger age groups, *i.e.* for those born starting from 1991/92 in comparison with their peers born 11 years earlier (Figure 4).

**Table 13.** Associations of different variables with *H. pylori* seropositivity according to multiple logistic regression analysis

| Variable | OR (95% CI) | *p*-value |
|---|---|---|
| Year of enrolment | | <0.001 |
| 1991 | 1.92 (1.33 – 2.76) | |
| 2002 | 1.00 | |
| Age | 1.17 (1.12 – 1.22)* | <0.001* |
| Gender | | NS |
| boy | 1.00 | |
| girl | 1.07 (0.77 – 1.49) | |
| Region of residence | | NS |
| city and county of Tartu | 1.00 | |
| adjacent counties | 0.93 (0.56 – 1.57) | |
| more distant counties | 1.33 (0.92 – 1.93) | |
| Place of residence | | NS |
| urban | 1.00 | |
| rural | 1.03 (0.73–1.45) | |
| Diagnosis | | NS |
| recurrent abdominal pain | 1.07 (0.55 – 2.06) | |
| acute intestinal infections | 1.07 (0.55 – 2.06) | |
| epilepsy | 0.93 (0.38 – 2.26) | |
| other | 1.00 | |

*Corresponding to the one-year age difference

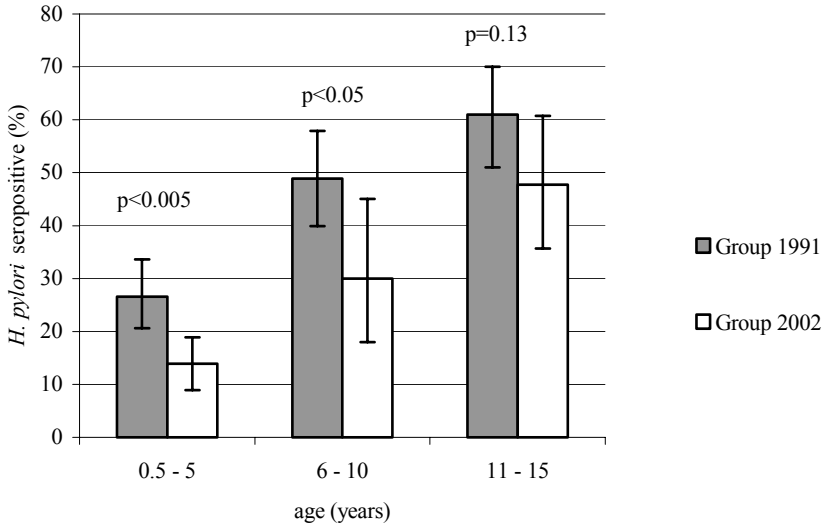


**Figure 4.** Age-standardized *H. pylori* seroprevalence rates in different age groups

## 7.3. Long-term recurrence rate after treatment of *H. pylori* infection (paper III)

Before treatment, all 27 children had *H. pylori*-positive chronic antral gastritis and a positive rapid urease test result; 19 of them had also chronic corpus gastritis.

A total of 23 children attended the 1st follow-up visit 4–6 weeks after the completion of anti-*H. pylori* treatment; all but one received triple treatment. Eighteen of them (78%, 95% CI 56–92%) appeared to be *H. pylori* negative on the basis of both histological examinations and the rapid urease test, in five children eradication therapy failed as they were *H. pylori* positive according to both histology and the rapid urease test.

Sixteen out of the 18 patients who were tested negative for *H. pylori* infection at the 1st follow-up visit attended the 2nd follow-up visit in 1997, mean 17.8±7.1 months after treatment; one of them turned out to be *H. pylori*-positive. In 2002, all 16 patients came to the 3rd follow-up visit mean 6.6±0.8 years after treatment, and 5 out of 15 previously *H. pylori*-negative patients had become *H. pylori*-positive since the 2nd follow-up visit. The *H. pylori* status of the previously *H. pylori*-positive patient did not change during the last follow-up period. The recurrence rates (% per patient-year) were calculated for the periods between the follow-up visits and for the whole follow-up period (Table 14), and they were 4.2% for the period between the 1st and the 2nd follow-up visit, 7.6% (95% CI 2.5–17.6%) for the period between the 2nd and the 3rd follow-up visit and 6.7% (95% CI 2.5–14.5%) for whole follow-up. Cumulative recurrence rate was 37.5% (95% CI 15.2%–64.6%) at the end of this study.

**Table 14.** Recurrence rates during different periods of follow-up

| Follow-up period | No of recurrences | Sum of follow-up time (years) | Recurrence rate (% per patient-year) |
|---|---|---|---|
| Between 1st and 2nd follow-up visit | 1 | 23.7 | 4.2% |
| Between 2nd and 3rd follow-up visit | 5 | 66.1* | 7.6% |
| Whole follow-up | 6 | 89.8* | 6.7% |

* Calculated on the basis of the assumption that the recurrence cases were equally distributed throughout the mean follow-up period of 5.1 years since the 2nd follow-up visit

Of the patients tested *H. pylori* positive at the 1st follow-up visit, all 5 attended the subsequent visits. One of them turned out to be *H. pylori* negative at the 2nd follow-up visit and was *H. pylori* negative also in 2002. The remaining four

patients were *H. pylori* positive during the 2nd and the 3rd follow-up visits. In addition, two patients who missed the 1st follow-up visit attended the subsequent visits, at which time they proved to be *H. pylori* positive.

Of the 27 patients who had received treatment *per protocol* in 1993–95, 23 (85%) were followed up until 2002. Eleven of them (48%, 95% CI 27–69%) remained *H. pylori* negative. The data about the *H. pylori* status over time for all patients in whom *H. pylori* eradication treatment was intended during 1993–1995 are shown in Table 15.

No borderline $^{13}$C-UBT results were recorded during this study.

**Table 15.** The number of *H. pylori*-positive patients and *H. pylori*-negative patients, and the number of recurrences and spontaneous eliminations of the infection over time

| | Total No. of patients | No. of *H. pylori* positive patients | No. of *H. pylori* negative patients | No. of recurrences | No. of spontaneous eliminations |
|---|---|---|---|---|---|
| Intention to treat (in 1993–1995) | 34 | 34 | 0 | – | – |
| Treatment *per protocol* (in 1993–1995) | 27 | 27 | 0 | – | – |
| 1st follow-up visit (4–6 w after treatment) | 23 | 5 | 18 | – | – |
| 2nd follow-up visit (in 1997) | 23 | 7* | 16 | 1 | 1 |
| 3rd follow-up visit (in 2002) | 23 | 12* | 11 | 5 | 0 |

*Two of the patients did not attend the 1st follow-up visit.

No association was found either between the recurrences or between the *H. pylori* status and the following factors: age, gender, diagnosis at the beginning of the study (duodenal ulcer *versus* non-ulcer recurrent abdominal pain), residence in urban/rural area, size of family, presence of young children (<5 years) in family, crowding (number of persons divided by number of living rooms less than 1 *versus* 1 and more), type of water supply (absence or presence of running water or of hot water supply), education of parents and keeping of pets or farm animals. The only statistically significant difference was found in antibiotic usage: nine out of 12 *H. pylori* positive patients *versus* two out of 11 *H. pylori* negative patients had used antibiotics for various reasons during one year before the 3rd follow-up visit ($p$=0.01).

## 7.4. The effect of administration of bovine immune colostrum on *H. pylori* infection and on chronic gastritis (paper IV)

All 20 children enrolled in the study had *H. pylori*–positive chronic gastritis. Within 2 weeks after the discontinuation of treatment, 16 children attended a check-up visit. Compliance was good: 14 children admitted that they had taken all dosages of specific immune colostrum, two children missed 2 and 3 dosages, respectively. No side effects were recorded. A majority of children, 14 out of 16, admitted symptomatic improvement since the beginning of treatment: 5 did not have any abdominal pain, 9 had less abdominal pain; 2 children felt no changes.

**Table 16.** The results of upper gastrointestinal endoscopy, rapid urease test and histological examination before and within 2 weeks after the discontinuation of BIC administration

| | | Before treatment n=20 | After treatment n=16 | *p*-value |
|---|---|---|---|---|
| Endoscopic features | antral nodularity | 11 | 9 | NS |
| | normal | 9 | 7 | |
| Rapid urease test | positive | 20 | 16 | NS |
| | negative | 0 | 0 | |
| Histological examination of the corpus mucosa | *H. pylori* colonization density | | | NS |
| | 3 (severe) | 8 | 3 | |
| | 2 (moderate) | 5 | 4 | |
| | 1 (mild) | 4 | 6 | |
| | 0 (none) | 3 | 3 | |
| | chronic inflammatory infiltration | | | NS |
| | 3 (severe) | 3 | 0 | |
| | 2 (moderate) | 0 | 1 | |
| | 1 (mild) | 10 | 7 | |
| | 0 (none) | 7 | 8 | |
| | epithelial degeneration | | | NS |
| | present | 8 | 5 | |
| | not present | 12 | 11 | |
| Histological examination of antrum mucosa | *H. pylori* colonization density | | (n=14)[a] | |
| | 3 (severe) | 12 | 5 | NS |
| | 2 (moderate) | 5 | 4 | |
| | 1 (mild) | 2 | 5 | |
| | 0 (none) | 1 | 0 | |
| | chronic inflammatory infiltration | | | |
| | 3 (severe) | 8 | 0 | <0.05[b] |
| | 2 (moderate) | 2 | 4 | |
| | 1 (mild) | 9 | 8 | |
| | 0 (none) | 1 | 2 | |
| | epithelial degeneration | | | |
| | present | 15 | 9 | NS |
| | not present | 5 | 5 | |

[a] in 2 cases histological examinations of the antrum mucosa were not performed owing to technical failure

[b] between those with grade 3 and grade 0–2 (Fisher's exact test); difference remained statistically significant when only patients with both pre- and post-treatment antrum biopsy samples (n=14) were taken into account

According to the post-treatment histological examinations and the rapid urease test, none of the patients showed eradication of *H. pylori* infection; however, there was noted a tendency towards improvement of chronic gastritis (Table 16).

Comparison of the results of the histological examinations of the individual patients' revealed that unidirectional changes both in antrum and corpus mucosa in some or all histological variables occurred in 9 patients: of them in 7 cases towards recovery and in 2 cases towards deterioration; there was no case with opposite changes (Table 17).

**Table 17.** The results of the histological examination before and after BIC administration (recovery of the histological variables after treatment is indicated by light grey background, deterioration by dark grey background)

| Case no. | Antrum mucosa | | | | | | Corpus mucosa | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *H. pylori* colonization | | chronic inflammatory infiltration | | epithelial degeneration | | *H. pylori* colonization | | chronic inflammatory infiltration | | epithelial degeneration | |
| | B* | A** | B* | A** | B* | A** | B* | A** | B* | A** | B* | A** |
| **1** | 3 | – | 3 | – | yes | – | 0 | 0 | 0 | 0 | no | no |
| **2** | 3 | 3 | 3 | 2 | yes | yes | 3 | 3 | 1 | 1 | yes | yes |
| **3** | 3 | 1 | 3 | 1 | yes | no | 3 | 1 | 3 | 1 | yes | no |
| **4** | 3 | 2 | 3 | 1 | yes | no | 2 | 0 | 1 | 0 | no | no |
| **5** | 3 | 1 | 3 | 0 | yes | no | 1 | 0 | 0 | 0 | no | no |
| **6** | 3 | 1 | 1 | 1 | yes | yes | 3 | 2 | 1 | 1 | no | no |
| **7** | 2 | 2 | 3 | 1 | yes | no | 1 | 1 | 1 | 0 | no | no |
| **8** | 3 | – | 3 | – | yes | – | 3 | 3 | 1 | 1 | yes | yes |
| **9** | 2 | 3 | 1 | 2 | yes | yes | 3 | 3 | 1 | 1 | yes | yes |
| **10** | 3 | 1 | 1 | 0 | no | no | 1 | 1 | 0 | 0 | no | no |
| **11** | 3 | 3 | 2 | 2 | yes | yes | 2 | 2 | 0 | 0 | yes | yes |
| **12** | 3 | 1 | 1 | 1 | yes | yes | 3 | 1 | 1 | 1 | yes | yes |
| **13** | 3 | 2 | 1 | 1 | yes | yes | 2 | 2 | 1 | 0 | yes | no |
| **14** | 0 | 3 | 0 | 1 | no | yes | 1 | 1 | 0 | 1 | no | no |
| **15** | 2 | 2 | 1 | 1 | yes | yes | 2 | 2 | 0 | 0 | no | no |
| **16** | 1 | 3 | 2 | 2 | no | yes | 1 | 1 | 1 | 2 | no | no |

* before administration of BIC

** after administration of BIC

*H. pylori* colonization and chronic inflammatory infiltration was graded as follows: 0 – none, 1 – mild, 2 – moderate, 3 – severe, "–" grading was not performed owing to technical failure

# 8. DISCUSSION

## 8.1. Methodological considerations

### 8.1.1. Study designs, subjects and settings

To determine the prevalence of *H. pylori* infection in children living in Southern Estonia, a community-based study was carried out (paper I). The subjects in this study were randomly selected schoolchildren living in five Southern Estonian counties. Of the children of Estonia approximately 23% lives in these counties (Statistical Office of Estonia 2001). As all children in Estonia up to age 16 are subjects of the compulsory basic education, the prevalence rate found for schoolchildren reflects the situation in the general population. Altogether 421 sera were available for the *H. pylori* serosurvey. The size of the sample was adequate to assess prevalence rate in the given age group of children in general, taking into account the range of the 95% confidence interval. The study had less than optimal power, <80%, to detect differences between certain ages.

For the evaluation of the dynamics of the prevalence of *H. pylori* infection in children in Estonia 1991–2002, a hospital-based study was carried out (papers II, V). The observed decrease in the seroprevalence of *H. pylori* infection may not have reflected the changes at the level of general population if some selection bias occurred in hospitalization practices, so that patients with a higher prevalence of *H. pylori* infection were more likely to be hospitalized in 1991 than in 2002.

The prevalence of *H. pylori* infection increases with age (Pounder & Ng 1995). As the age distribution of the patients was different in Groups 1991 and 2002, age was included as a variable in multiple logistic regression analysis and age-standardized prevalence rates of *H. pylori* infection were calculated for the both study groups.

One of the theoretical possibilities for the selection bias could be that some changes occurred in the indications for hospitalization so that patients with *H. pylori*-associated diseases were more likely to have been hospitalized in 1991 than in 2002. There was no single patient with the main *H. pylori*-associated disease, peptic ulcer, in either study group. In Group 1991, the proportion of children with recurrent abdominal pain, was significantly higher than in Group 2002. Recurrent abdominal pain has been found to be associated with *H. pylori* infection in some studies, however most community-based epidemiological studies have failed to establish that *H. pylori* infection is more prevalent in children with recurrent abdominal pain than in children without it (O'Donohoe *et al.* 1996, Macarthur *et al.* 1999, Bode *et al.* 1998b, Bode *et al.* 2003). In hospital-based studies, no association between *H. pylori* infection and recurrent abdominal pain has been found when children with peptic ulcer were

excluded (van der Meer *et al.* 1992b, McCallion *et al.* 1995, Wewer *et al.* 1998). Nor was any association between *H. pylori* infection and recurrent abdominal pain found either in the present study. In Group 2002, there were more children with acute intestinal infections and epilepsy than in Group 1991. Some studies have found that children with *H. pylori* infection have more acute intestinal infections (Clemens *et al.* 1995, Passaro *et al.* 2001, Shmuely *et al.* 2004). On the contrary, some studies have found that *H. pylori*-positive subjects have less diarrheal diseases (Rothenbacher *et al.* 2000b, Bode *et al.* 2001, Perry *et al.* 2004) and some have not found any such association at all (Isenbarger *et al.* 1998, Rahman *et al.* 1998). In our study, acute intestinal infections were not associated with *H. pylori* serostatus, either when we analyzed the study groups separately or together. In fact, the higher number of patients with acute intestinal infections in Group 2002 does not reflect the actual increase in hospitalization over time, as 342 and 320 patients with this diagnosis were hospitalized in 1991 and 2002, respectively, which accounted for 8.9% and 8.7% of all hospitalizations in those years ($p>0.05$). The difference between the study groups can be explained by the relatively high drop-out rate due to technical reasons for Group 1991: as majority of these patients were suffering from dehydration and were aged under 5 years, the amount of the serum was relatively small and had been initially exploited for a screening study of celiac disease in many cases. A recent single report suggested an association between *H. pylori* infection and idiopathic epilepsy (Okuda *et al.* 2004); this association was not found in our study.

Another theoretical possibility could be that hospitalization was less accessible to children from lower socio-economic conditions in 2002 compared with 1991. Actually, hospital care was completely free of charge for all children in Estonia both in 1991 and 2002.

A selection bias may have occurred due to regional changes or urban/rural disparity in hospitalization practices over time, if the prevalence of *H. pylori* infection differed in different places of residence. In the current study, we did not find that such changes occurred 1991–2002.

Although the possibility that the results of the study might have been influenced by some selection bias cannot be ruled out completely, we could not identify any possible reason for the decrease in the seroprevalence of *H. pylori* infection over time other than the fact that it reflects similar changes occurring in general population.

For determination of the post-treatment recurrence rate of *H. pylori* infection, a prospective follow-up study was carried out (paper III). Despite the long follow-up period, mean 6.6 years after treatment, participation rate was high: 85%, or 23 out of 27 patients attended the follow-up visits. In this study, we aimed to evaluate the possible risk factors for recurrence of *H. pylori* infection, however, the power of the study was low, less than 80%, to draw relevant conclusions.

In a study evaluating the effectiveness of the administration of the bovine immune colostrums (paper IV), the participation rate was also high: 80% of the enrolled children, or 16 out of 20, attended the check-up visit. Although the symptomatic improvement was noted in a majority of them, the follow-up period was short and the placebo effect may have largely contributed it. To provide more consistent conclusions and to evaluate the role of a possible placebo effect on symptomatic improvement, a placebo-controlled study should be carried out.

### 8.1.2. Methods of detection of *H. pylori* infection

#### 8.1.2.1. Serological tests

The ELISA used in children needs to be validated for pediatric population. When the cut-off values validated for adults are used, the specificity of the test in children is high, above 95% (Kindermann *et al.* 2001). However, low sensitivity of some tests in children less than 9–12 years of age has been reported (Raymond *et al.* 1996, Khanna 1998, de Oliveira *et al.* 1999b, Kindermann *et al.* 2001). When an adjusted cut-off level for pediatric patients was used, which is normally lower than for adults, ELISA was found to have an accuracy over >90% (Sunnerstam *et al.* 1999, Tindberg *et al.* 2001b). For the determination of the cut-off value, different methods can be used, mostly by the construction of the receiving operating characteristic curve (Fauchère 1996). In the present study, the cut-off value for seropositivity for ELISA was derived from the mean value of RAA+2SD of the sera obtained from children without gastritis or *H. pylori* infection (Brown & Swanson Beck 1989). The use of lower or higher cut-off values would not substantially influence the study results: in study I, the cut-off values of 20 and 30, would yield a seroprevalence rate of 59% and 54%, respectively.

In study II, the aim was to compare the *H. pylori* seroprevalence rates in different groups. To enhance the precision of the results we used immunoblot analysis in cases of borderline ELISA results. Immunoblot has been demonstrated to have high accuracy for pediatric population (Rocha *et al.* 2000, Raymond *et al.* 2000, Tindberg *et al.* 2001b), and it has been particularly useful in cases with doubtful ELISA results in children (Raymond *et al.* 2000). In this study ELISA and immunoblot showed good correlation: concordant results were obtained in 63 out of 87 (72%) borderline ELISA cases. As we performed serological analysis for the groups using the same methods, there could be no significant differences in the calculated adjusted odds ratios irrespective of the fact if the sensitivity and/or specificity of the method were low or high.

Frozen-stored sera were used in these studies. In study II, the sera of the patients of Group 1991 were stored for 11 years, and the sera of the patients of Group 2002 for a few months. Several epidemiological studies have

demonstrated high stability of the results of serological tests even after long-term storage of frozen sera (Kosunen *et al.* 1997, Gause-Nilsson *et al.* 1998). In the present study, no statistically significant differences were established between Group 1991 and 2002 when the mean RAA values of the *H. pylori*-positive sera were compared, indicating a high degree of IgG antibody stability despite the long-term storage of the sera. In addition, there was a high concordance between the ELISA results and the results of histological examinations for Group 1991.

#### 8.1.2.2. Histological examinations, rapid urease test, $^{13}$C-urea breath test

Histological examinations, rapid urease test as well as $^{13}$C-UBT have all served as the excellent methods for diagnosing *H. pylori* infection in children, with the diagnostic accuracy higher than 95% (Ni *et al.* 2000, Bazzoli *et al.* 2000, Kato *et al.* 2002). However, for the age group younger than six years the specificity of $^{13}$C-UBT can be lower (Kindermann *et al.* 2000). In the current study, none of the patients in whom $^{13}$C-UBT was performed belonged to this age group.

Both histological examinations and rapid urease test were used to determine the patients' pre-and post-treatment *H. pylori* status (papers III, IV), and they gave concordant results in all cases, therefore making the misclassification of the patients according to their *H. pylori*-status highly unlikely.

$^{13}$C-UBT was used for detecting recurrent *H. pylori* infections (paper III), as has been general practice in other similar studies (Kato *et al.* 1998, Rowland *et al.* 1999, Feydt-Schmidt *et al.* 2002). Slightly different cut-off values have been used for $\delta\ ^{13}$C in different studies, from 3.5‰ to 5.3 ‰, depending on the dose of $^{13}$C-urea, the type of the test meal, and the time of breath collection (Bazzoli *et al.* 2000, Kindermann *et al.* 2000, Kato *et al.* 2002). However, in our study not a single $\delta\ ^{13}$C-UBT fell in the range of 3.5–5‰.

The study of the effectiveness of administration of bovine immune colostrum was conducted as an open trial: there was no placebo group. Therefore it may be argued that the detected changes in the grades of *H. pylori* colonization and inflammatory infiltration were due to sampling error, as *H. pylori* and inflammation may have a patchy distribution in the gastric mucosa. For minimizing sampling error, two biopsy samples were taken both from the antrum and corpus mucosa. In all cases when changes were noted in the corpus mucosa, unidirectional changes in some histological variables occurred for the antrum mucosa, while no opposite changes were noted for the antrum and corpus mucosa, which renders sampling error as the main reason for the detected changes unlikely.

## 8.2. Prevalence of *H. pylori* infection among children aged 9–15 years and living in Southern Estonia (paper I)

The prevalence rate of *H. pylori* infection in children aged 9–15 years, living in Southern Estonia in 1993–1996, was 56% (95% CI 51–61%). This prevalence rate is comparable to the seroprevalence rates of the infection of 52% and 55% among 10–14-year-old children in St. Petersburg, Russia, and in Mexico, respectively (Malaty *et al.* 1996a, Torres *et al.* 1998), and to the seroprevalence rate of 56% among 14–17-year-old children in Siberian part of the Russia (Reshetnikov *et al.* 2003). A somewhat lower prevalence of the infection was found in a Latvian study, which was conducted a few years later, in 1998–1999: the prevalence rate of *H. pylori* infection in children aged 9–12 years was 32% (95% CI 17–51%). A significantly lower prevalence of the infection, well below 30%, has been reported for schoolchildren in Western European countries (Patel *et al.* 1994, De Giacomo *et al.* 2002, Heuberger *et al.* 2003). A marked, more than a 10-fold difference in seroprevalence rates between children from Estonia and from Sweden and Finland was found: in Sweden 3% of 11-year-olds and in Finland 4% of 14–15 year-olds were seropositive in 1994–1995 (Granström *et al.* 1997, Rehnberg-Laiho *et al.* 1998). Sweden and Finland are geographically very close to Estonia, but their historical background has been considerably different since 1940 when Estonia was incorporated in the Soviet Union. Therefore there were differences in the socioeconomic and living conditions between these countries.

In the present study it was found that within the same geographic area, children living in rural areas had significantly higher prevalence of *H. pylori* infection than children living in urban areas. While the data from a number of studies showed no difference in the prevalence of the infection between rural and urban areas (Mégraud *et al.* 1989, Fiedorek *et al.* 1991, Torres *et al.* 1998, Herbarth *et al.* 2001), a higher prevalence of the infection was found in urban areas in China and in West Virginia, USA (Mitchell *et al.* 1992a, Elitsur *et al.* 1998). In agreement with the current study, a higher prevalence of the infection was found in rural areas in Italy (Dore *et al.* 2002). These data indicate that risk for acquisition of *H. pylori* infection may be different in different settings.

In Estonia, there exist differences in the availability of different conveniences in dwellings between urban and rural areas. In 2000, 96%, 89% and 82% of the inhabitants of urban areas were living in dwellings with water supply system, flush toilet and hot water, respectively; the respective figures for the inhabitants of the rural areas were 70%, 52% and 49% (Statistical Office of Estonia 2004). Therefore the higher prevalence of the infection for those living in rural areas is consistent with faecal-oral and/or waterborne transmission of the infection.

## 8.3. Dynamics of the prevalence of *H. pylori* infection in children in Estonia 1991–2002 (papers II, V)

The prevalence of *H. pylori* infection decreased significantly among children during the period from 1991 to 2002: the age-standardized prevalence rate decreased by about one-third, from 42.2% to 28.1%. According to age-stratified analysis, a statistically significantly lower seroprevalence was noted for children born starting from 1991/92 compared with their peers born 11 years earlier.

Considerable decrease in the prevalence of *H. pylori* infection in successive birth cohorts has been observed in many Western European countries in the second half of the 20$^{th}$ century (Table 3). In Estonia, non-existent or only a marginal birth cohort effect was evident in the case of adults throughout the last century: no statistically significant differences were found between the age groups in retrospective histology-based studies of the adult general population in 1972 and 1979 (Maaroos 1995). A seroepidemiological study, carried out in 1990, established a slight decrease, from 89% to 83% (p=0.04), in the prevalence rate among the subjects belonging to the age groups 30–39 and 20–29 years; among the persons aged 30–79 years, the prevalence of the infection was uniformly high for all decades of life, ranging 89–94% (Vorobjova *et al.* 1994). A very high prevalence of *H. pylori* infection in young adults, >80%, has also been observed in other Eastern European countries (Matysiak-Budnik *et al.* 1996, Malaty *et al.* 1996a).

The disappearance of *H. pylori* from the Western countries has been associated with the continuous steady improvement of socioeconomic conditions: decreased transmission due to the improved living conditions and better hygiene and/or increased loss of the infection due to the increased usage of antibiotics are implicated as the main reasons (Parsonnet 2000). In Korea, where the prevalence of the infection among adults is high irrespective of the social class, lower prevalence was found among children of families of higher socioeconomic status (Malaty *et al.* 1996b).

Since 1991, profound social and economical changes such as transition to democratic polity and market economy have taken place in Estonia. The changes accompanied with a decrease in the gross domestic product in 1991–1994 and a growth thereafter (Kask *et al.* 2002). The country as a whole has been successful in restructuring its economy towards a liberal market economy. However, the changes in the society were not necessarily accompanied with the improvement of the socioeconomic conditions on the individual's level, as the stratification of the society on the richness-poverty scale has remarkably increased. As the data about the socioeconomic conditions of the patients' families are not available, we do not know if the decrease in the prevalence occurred predominantly among children from families of high socioeconomic status, as it was demonstrated in South Korea (Malaty *et al.* 1996b). We can

only speculate, on the basis of the national censuses of 1989 and 2000 and other nationwide statistics, which changes in the society may have played a role in the decreasing prevalence of *H. pylori* infection among children in Estonia. The average real terms income increased but not very markedly: by the end of the 1990s it was 1.2 times higher than in 1992 (Kask *et al.* 2002). There were no dramatic changes in the living conditions in Estonia in the 1990s, as in 2000 only 4.8% of the population lived in dwellings built in 1991 or later (Statistical Office of Estonia 2004). In 1989, 83% of the inhabitants had a water supply system in their dwellings (data from the Census of 1989, Statistical Office of Estonia, personal communication); in 2000 the respective figure was 88% (Statistical Office of Estonia 2004). The microbiological quality of water from the central supply system was good during the whole study period (Saava A, Dept of Public Health, University of Tartu, personal communication). Personal hygiene and food processing hygiene has apparently improved over time in Estonia, as the number of registered gastrointestinal infections declined steadily in the 1990s (Jõgiste *et al.* 2003).

The widespread use of antibiotics in children may also have contributed to the decreased prevalence. Even when the probability of eradication of *H. pylori* infection after a treatment course with a single antibiotic is low (Laheij *et al.* 1999), repeat antibacterial courses in childhood may theoretically have a substantial effect on the prevalence of *H. pylori* infection. However, the gradual decrease in the prevalence of *H. pylori* infection dates back to early 20$^{th}$ century, before the introduction of antibiotics (Kosunen *et al.* 1997, Gause-Nilsson *et al.* 1998). It is unclear whether the changes in antibiotic usage could have played a role in the changes in the prevalence of the *H. pylori* infection among children in Estonia. The data based on the whole population revealed no significant differences in the quantities of the prescribed antibiotics in 1989 in comparison with Nordic countries (Kiivet *et al.* 1992). A decrease in antibiotics prescription occurred in the middle of the 1990s (Kiivet *et al.* 1998).

Major changes occurred in the dietary habits of inhabitants of Estonia since 1991, as the deficit of many food products in shops was replaced by their all-the-year-round great variety. Over half of the Estonian population adopted healthier eating habits (Laur 2002). One of the most prominent changes was the abrupt growth in the consumption of fresh vegetables and fruits (Kasmel 2002). Whether individual susceptibility to *H. pylori* could be influenced by diet is an interesting but generally poorly studied issue. Some findings from cross-sectional studies suggest that higher intake of vitamin C and other micronutrients is protective against *H. pylori* infection. In a Colombian study of children aged 2–9 years, increased intake of dietary vitamin C and β-carotene was associated with decreased prevalence of the infection, while the effects persisted after adjustment for indicators of socioeconomic status, household crowding and hygiene (Goodman *et al.* 1997). The same associations were shown for adults in the USA (Fontham *et al.* 1995). Begue *et al.* (1998) found inverse association between consumption of fruits and *H. pylori* seroprevalence

among Peruvian children. However, in a large study among Mexican public school students, intakes of antioxidants (vitamin C, vitamin E, and carotenes), fruits, and vegetables were not statistically associated with *H. pylori* sero-prevalence (Constanza *et al.* 2004). The limitation of these studies is that dietary information was related to current practices, which might not be pertinent to an infection acquired at some time in the past. Some *in vitro* and animal studies have also demonstrated the inhibitory effect of vitamin C and other antioxidants on the growth of *H. pylori* (Zhang *et al.* 1997, Wang *et al.* 2000, Sjunnesson *et al.* 2001, Tabak *et al.* 2003). To the best of my knowledge, there are no published data about the impact of food preservatives on *H. pylori* infection.

## 8.4. Long-term recurrence rate after treatment of *H. pylori* infection (paper III)

Our study revealed a higher recurrence rate of *H. pylori* infection, 6.7% (95% CI 2.5–14.5%), in children and adolescents compared with the results of the studies conducted in Western Europe and Japan. In Germany, where the prevalence rate is less than 10% among persons up to 20 years of age (Herbarth *et al.* 2001), the recurrence rate for children aged 1.8–18 years was 2.3% per patient-year (Feydt-Schmidt *et al.* 2002). In Japan, where the prevalence of *H. pylori* among 10–16-year-old persons was 26.0% (Miyaji *et al.* 2000), the recurrence rate for children aged 5 to 16 years was 2.4% per patient-year (Kato *et al.* 1998). A higher recurrence rate, 5.8% per patient-year, was found in a study conducted in Ireland (Rowland *et al.* 1999), while the prevalence of the infection among the parents and siblings was >65%; however, for children older than 5 years the recurrence rate was 2.0% per patient-year. In our study, even when we assumed that four patients who were lost to follow-up were successfully treated and remained *H. pylori* negative during a mean of 6.6 years after treatment, the recurrence rate would be still quite high, 4.8% per patient-year. A high recurrence rate was found also in an Estonian study conducted in adults: six recurrences were observed in 57 successfully eradicated patients during one-year follow-up (Maaroos *et al.* 2001), which yields a recurrence rate of 10.5% per patient-year.

Higher recurrence rates within the first 12 months after treatment have been observed in some studies (Xia *et al.* 1997, Gisbert *et al.* 1998), but were not found in our study. It is suggested that the higher recurrence rates noted during the first post-treatment year are actually due to recrudescences of partially suppressed infection but not real reinfection (Bell *et al.* 1996, Gisbert *et al.* 1998, Hildebrand *et al.* 2001). Although we did not perform molecular analysis,

recrudescence of suppressed infection is unlikely to explain the high recurrence rate, as most recurrences occurred after the 2nd follow-up visit.

Little is known about the factors, which may facilitate reinfection. The number of recurrence cases in the studies is low, exceeding rarely 10 cases per study; hence the power of analysis has been generally too low to accurately detect risk factors. In adults, older age was found to be a protective factor (Gisbert *et al.* 1998, Soto *et al.* 2003). In a study conducted in Ireland, the risk of reinfection was found to be higher for children less than 5 years of age (Rowland *et al.* 1999), however, other pediatric studies have not confirmed this finding (Feydt-Schmidt *et al.* 2002, Farrell *et al.* 2004). In our study we did not find any statistically significant associations between different variables and either *H. pylori* status or recurrences, except when antibiotics have been used. Surprisingly, we established that before the last follow-up visit antibiotics had been used more often for various reasons by the *H. pylori*-positive patients compared with the *H. pylori*-negative patients. Antibiotic usage has been generally associated with the higher probability of spontaneous clearance of the infection in children (Rothenbacher *et al.* 2002a). However, the probability of an antibacterial treatment course, prescribed for other purposes, to clear *H. pylori* infection is low (Leung *et al.* 2002). In our study the patient who was spontaneously cleared of *H. pylori* infection after an unsuccessful antibacterial treatment course denied any use of antibiotics during follow-up.

According to clinical guidelines on *H. pylori* infection in children, eradication is recommended for *H. pylori*-positive children who have duodenal or gastric ulcer disease (Sherman *et al.* 1999, Drumm *et al.* 2000, Gold *et al.* 2000) and for children in whom *H. pylori* infection was identified at endoscopic examination (Sherman *et al.* 1999, Drumm *et al.* 2000).

There is no widely accepted screening and treatment programme for *H. pylori* infection in asymptomatic individuals (Forman & Graham 2004, Correa *et al.* 2004, Sullivan *et al.* 2004). Although such a programme may have the potential to reduce the incidence of peptic ulcer and gastric cancer and might be cost-effective (Parsonnet *et al.* 1996, Roderick *et al.* 2003, Leivo *et al.* 2004), there is some disagreement in this respect (Forbes & Threlfall 1998). Before population screening for *H. pylori* infection and a subsequent eradication programme can implemented, potential benefits and risks for the given population (taking into account the prevalence of infection, risk of gastric cancer, effectiveness of treatment, risk of reinfection) should be carefully weighted, and widespread support from community should be gained to achieve high compliance rates (Forman & Graham 2004). The risks include side effects of drugs, the risk of generating resistant strains of *H. pylori* and other bacterial species (Huang *et al.* 1999, Bontems *et al.* 2001, Sjölund *et al.* 2003) as well as potential psychological morbidities, *e.g.* anxiety among those who fail therapy. The issue whether *H. pylori* eradication is or is not associated with oesophageal cancer has to be fully resolved before implementing such a programme (Forman & Graham 2004).

The present study revealed that a significant decrease in *H. pylori* prevalence in children has occurred during past 11 years in Estonia: the odds of being *H. pylori* seropositive were 1.92 times higher in 1991 than in 2002. The data obtained from the same background population for approximately the same time period allowed us to made hypothetical calculations for two scenarios: first, if antibacterial treatment is intended for administration to all 11–15 year-old *H. pylori*-positive children in population, the prevalence rate of the infection in the target group would be 50% (it was 47.7% in the age group of 11–15 years in 2002), compliance and effectiveness would both be 80%, and reinfection rate would be 6% per patient-year. In this case, the prevalence rate of *H. pylori* infection among these children would be 18% after treatment, and 34% after 11 years. According to the other scenario, owing to the birth cohort effect, there occurs only a natural decrease in *H. pylori* prevalence. In this case, it can be assumed that at the starting point, the odds for *H. pylori* positivity would be 1.92 times higher than the odds 11 years later. Then, after 11 years the prevalence rate for the age group of 11–15 years would drop from 50% to 34% too. According to these hypothetical calculations, it can be concluded that the spontaneous disappearance of *H. pylori* infection in children since 1991 in Estonia has been so rapid that it is comparable to the result of an extensive treatment programme of this infection.

## 8.5. The effect of administration of bovine immune colostrum on *H. pylori* infection and on chronic gastritis (paper IV)

A tendency towards recovery from gastritis and/or decrease in colonization degree was noticed in 9 out of 16 patients within 2 weeks after discontinuation of administration of BIC, however, no eradication of the infection was achieved. Some studies have shown that in animal experiments and in some cases in humans, eradication can be achieved by orally administered BIC. The overall cure rate of *H. pylori* infection was 66% in mice after 10–20 days of BIC treatment (Casswall *et al.* 2002). The same authors reported the eradication of the infection in 2 out of 8 adult patients with BIC administered 4 g/day for 28 days (Casswall *et al.* 2002). However, no eradication was detected by means of $^{13}$C-UBT in placebo-controlled double-blind study of 24 *H. pylori*-positive infants receiving 1g of BIC/day for 30 days (Casswall *et al.* 1998). No eradication of the infection was noted either in another study, when 15 adults received a short, 2 day-course with daily doses up to 15 g of BIC (Opekun *et al.* 1999). The histological status of the gastric mucosa was not assessed in these two studies.

It is not likely that the lack of the eradicative effect of BIC was due to the degradation of immunoglobulins in the human gastrointestinal tract. It has been shown that bovine IgG is relatively resistant to pepsin cleavage and that its immunologic activity is retained after passage through the gut (Hilpert *et al.* 1987, Roos *et al.* 1995). Possibly, higher doses or a longer treatment course of specific immune colostrum might lead to better results. The dose-dependent effect of oral bovine specific immunoglobulin has been shown previously in the treatment of rotaviral gastroenteritis (Hilpert *et al.* 1987). Lack of the sufficient therapeutic effectiveness of BIC may be caused by the fact that *H. pylori* is localized beneath the mucus layer, so that orally administered antibodies are unable to penetrate the mucus at sufficient concentrations and affect the infection. Our results are consistent with the finding that breast-feeding provides little protection against *H. pylori* infection (Gold *et al.* 1997, Thomas *et al.* 2004).

In our study, no side effects of the BIC were recorded. So far, no significant health risks have been reported during or after oral ingestion of immune milk preparations, and this approach is generally regarded as a non-invasive, and hence safe, intervention regime (Korhonen *et al.* 2000b). However, atopic reactions may occur in sensitized persons (Bernhisel-Broadbent *et al.* 1991).

# 9. CONCLUSIONS

1. The prevalence of *H. pylori* infection among children aged 9–15 years, living in Southern Estonia was 56%, which is significantly higher than among children of same age living in Scandinavian and Western European countries**.** *H. pylori* infection was more prevalent among children living in rural areas than among children living in urban areas.
2. The prevalence of *H. pylori* infection among children in Estonia decreased significantly after the reestablishment of the independence of Estonia in 1991, a period of profound social and economic changes: the age-standardized *H. pylori* seroprevalence rate decreased from 42.2% to 28.1% (p=0.0002) during 1991–2002, while the adjusted odds for *H. pylori* seropositivity decreased 1.92 (95% CI 1.33–2.76) times.
3. The recurrence rate of *H. pylori* infection after combined antibacterial treatment for children and adolescents in Estonia was 6.7% per patient-year, which is higher than the recurrence rates determined for their peers in Western Europe and Japan. Cumulative reinfection rate was 37.5% at the end of the follow-up of mean 6.6 years. The risk factors for recurrent *H. pylori* infection were not established.
4. Bovine immune colostrum did not eradicate *H. pylori* infection in children; however, there was noted a tendency for recovery from chronic gastritis.

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# SUMMARY IN ESTONIAN

## *Helicobacter pylori* infektsioon lastel: epidemioloogilisi ja ravi aspekte

*H. pylori* infektsiooni peetakse üheks levinumaks krooniliseks infektsiooniks maailmas. Hinnanguliselt on selle mikroobiga nakatunud vähemalt pool inimkonnast, samas on selle infektsiooni levimus maakera eri piirkondades erinev. Üldistatult on võimalik eristada kahte erinevat levimusmustrit. Lõuna-Ameerika, Aafrika ning paljudes Aasia riikides on *H. pylori* infektsioon kõigis vanuserühmades väga levinud, nakatunud on rohkem kui 50% lastest ning rohkem kui 80% täiskasvanutest, kusjuures levimusmäärad eri vanuses täiskasvanute hulgas on sarnased. Seevastu Lääne-Euroopas ning Põhja-Ameerikas on laste hulgas nakatunuid vähe, alla 20%; täiskasvanute hulgas aga on nakatunute osakaal vanemates vanuserühmades oluliselt suurem kui nooremates vanuserühmades. *H. pylori* infektsioon on levinum madalama sotsiaal-majandusliku staatusega inimeste hulgas.

Nakatumine *H. pylori* infektsiooni toimub peaaegu alati lapseeas. *H. pylori*-negatiivse täiskasvanu jaoks on risk nakatuda väga väike, umbes 0,5% inimaasta kohta, mis on ligikaudu võrdne infektsiooni spontaanse paranemise tõenäosusega *H. pylori*-positiivsel täiskasvanul. Arenenud tööstusmaades on risk nakatuda *H. pylori* infektsiooni viimastel aastakümnetel oluliselt vähenenud (sünnikohordi fenomen). *H. pylori* infektsioon levib inimeselt inimesele, peamiselt perekonnasisesi, tõenäoliselt fekaal-oraalsel või oro-oraalsel teel; võimalik, et ka saastunud joogivee ja toiduga. Nakatumise vähenemise põhjused pole täpselt teada, eelkõige peetakse põhjuseks inimeste paranenud elutingimusi ja hügieeni. Eestis, samuti ka Poolas ja Venemaal on *H. pylori* infektsioon täiskasvanute hulgas väga levinud, kusjuures ka täiskasvanute nooremates vanuserühmades on levimusmäär suurem kui 80%. Enne käesolevat uurimistööd oli teadmata, milline on *H. pylori* infektsiooni levimus laste hulgas Eestis, samuti puudusid andmed selle kohta, kas Ida-Euroopas (sh. Eestis) on alates 1990ndate aastate algusest aset leidnud suurte sotsiaal-majanduslike muutuste foonil *H. pylori* infektsiooni levimus muutunud.

*H. pylori* põhjustab kroonilist gastriiti. See mikroob võib nakatunutel põhjustada peptilist haavandit ja maovähki ning soodustada rauavaegusaneemia kujunemist. *H. pylori* infektsiooni raviks kasutatakse antimikroobsete ja maosoolhappe sekretsiooni pärssivate ravimite kombinatsioone, parimaid tulemusi annab vähemalt 3 ravimi kompleksne manustamine vähemalt 7 päeva jooksul. Selliste raviskeemide rakendamise tulemusel saavutatakse infektsiooni eradikatsioon 75–85% ravitutest. Raviga võivad kaasneda probleemid, eelkõige ravimite kõrvaltoimete teke ja mikroobide antibiootikumiresistentsuse kujunemine. Võimalik on ravijärgne taasnakatumine. Arenenud tööstusriikides on täiskasva-

nutel ravijärgse reinfektsiooni risk väike, üldiselt <2% inimaasta kohta. Reinfektsiooni risk on suurem arengumaades. Laste hulgas on ravijärgse reinfektsiooni riski vähe uuritud. Olemasolevad uurimused on läbi viidud piirkondades, kus laste hulgas on *H. pylori* levimus üldiselt väike, nimelt Lääne-Euroopas ja Jaapanis. Nende uurimistööde tulemusel arvutatud reinfektsioonikordajad jäävad vahemikku 2,0–2,4% inimaasta kohta.

*H. pylori* infektsiooni antibiootikumraviga seotud probleemide tõttu on uuritud *H. pylori* alternatiivse ravi võimalusi. Üheks selliseks võimaluseks on spetsiifiliste antikehade suukaudne manustamine. Kõrges tiitris spetsiifilisi antikehi sisaldab immuniseeritud veiste kolostrum. *H. pylori* surmatud rakkudega immuniseeritud veistelt saadud immuunkolostrum on *in vitro* uuringutes *H. pylori* suhtes bakteriotsiidse toimega, loomkatsetes on immuunkolostrumi manustamise järgselt saavutatud *Helicobacter spp.* eradikatsiooni. Kas spetsiifiline veise immuunkolostrum avaldab mõju *H. pylori* infektsioonile ja kroonilisele gastriidile inimestel, oli enne käesolevat uurimistööd uurimata.

## Uurimistöö eesmärgid

Käesoleva uurimistöö eesmärgiks oli uurida laste *H. pylori* infektsiooni epidemioloogilisi ja ravi aspekte. Uurimistöö kitsamad eesmärgid olid järgmised:

1. Määrata *H. pylori* infektsiooni levimus 9–15-aastaste Lõuna-Eestis elavate laste hulgas.
2. Hinnata *H. pylori* infektsiooni levimuse dünaamikat laste hulgas 11 aasta jooksul suurte sotsiaal-majanduslike muutuste perioodil, aastatel 1991–2002.
3. Määrata *H. pylori* reinfektsioonikordaja pärast kombineeritud antibakteriaalset ravi ning uurida reinfektsiooni riskifaktoreid lastel ja noorukitel Eestis.
4. Uurida üht alternatiivset ravivõimalust antibakteriaalsele ravile, spetsiifilise veise immuunkolostrumi manustamise mõju *H. pylori* infektsioonile ja kroonilisele gastriidile.

## Uuritavad ja meetodid

*H. pylori* infektsiooni levimuse määramiseks viidi läbi levimusuuring 421 juhuslikult valitud 9-, 12- ja 15-aastase Lõuna-Eesti koolilapse hulgas. *H. pylori* infektsiooni kindlakstegemiseks määrati vereseerumis spetsiifilisi IgG tüüpi antikehi ELISA meetodil.

*H. pylori* levimuse dünaamika hindamiseks lastel viidi läbi levimusuuringud aastatel 1991 ja 2002 samade valikukriteeriumite alusel moodustatud uurimisrühmade hulgas. Uuringusse arvati SA TÜK lastekliinikusse 1991. a. jaanuarist maini ja 2002. a. veebruarist maini hospitaliseeritud lapsed vanuses 6 kuud kuni 15 aastat, kel oli hospitaliseerimise käigus võetud veeniverd. 1991. a. moodustatud uurimisrühmas oli 425 last, 2002. a. moodustatud uurimisrühmas oli 296 last. *H. pylori* infektsiooni kindlakstegemiseks määrati vereseerumis spetsiifilisi IgG tüüpi antikehi ELISA meetodil, piirjuhtudel täpsustati tulemus

immunoblot-meetodil. Uurimisrühmade võrdlemiseks kasutati mitmest regressioonanalüüsi.

*H. pylori* reinfektsioonikordaja määramiseks lastel ja noorukitel viidi läbi prospektiivne uuring. Uuringusse kutsuti kõik patsiendid, kes olid aastatel 1993–1995 saanud SA TÜK lastekliinikus määratud *H. pylori* infektsiooni vastase ravikuuri (n=27, vanus 7–17 a.). Patsiendid kutsuti 1. järelkontrollile 4–6 nädalat pärast ravi lõppu, 2. järelkontrollile aastal 1997 (keskmiselt 18 kuud pärast ravi) ja 3. järelkontrollile aastal 2002 (keskmiselt 6,6 aastat pärast ravi). 1. järelkontrollil tehti seedetrakti ülaosa endoskoopiline uuring, mao antrumi ja korpuse limaskesta proovitükkide histoloogiline uuring ja ureaasi kiirtest, 2. ja 3. järelkontrollil kasutati *H. pylori* infektsiooni kindlakstegemiseks $^{13}C$ uurea hingamistesti. Enne ravi ja kõigil järelkontrollidel täideti küsimustik.

Hindamaks spetsiifilise veise immuunkolostrumi suukaudse manustamise mõju *H. pylori* infektsioonile ja kroonilisele gastriidile viidi läbi pilootuuring. *H. pylori* surmatud rakkudega immuniseeritud veistelt saadud immuunkolostrumi manustati kahekümnele korduvate kõhuvaluvaevustega ja *H. pylori*-positiivse kroonilise gastriidiga lapsele 21 päeva vältel. Kõik uuritavad kutsuti kontrollile 2 nädala jooksul pärast ravi lõppu. *H. pylori* kolonisatsiooni ja kroonilise gastriidi raskusastme hindamiseks enne ja pärast ravi tehti mao antrumi ja korpuse limaskesta proovitükkide histoloogilisi uuringuid ja ureaasi kiirteste.

## Uurimistöö peamised tulemused

*H. pylori* infektsiooni levimusmäär Lõuna-Eestis 9–15-aastaste laste hulgas oli 56% (95% CI 51–61%), mis on oluliselt kõrgem kui sama vanuserühma lastel Skandinaavias ning Lääne-Euroopa riikides. *H. pylori* infektsiooni levimus oli kõrgem maalaste hulgas võrreldes linnalastega: levimusšansside suhe oli 1,96 (95% CI 1,32–2,92).

Ajavahemikul 1991–2002 on *H. pylori* levimus laste hulgas oluliselt vähenenud: vanusele standarditud *H. pylori* infektsiooni levimusmäär oli 1991. aastal 42,2% ja 2002. aastal 28,1% ($p$<0,0002). *H. pylori* infektsiooni levimusšanss oli 1991.a. 1,92 (95% CI 1,33-2,76) korda suurem kui 2002.a.

*H. pylori* reinfektsiooni riski hindamiseks läbi viidud prospektiivse uuringu kontrollvisiitidel käis 23 patsienti (85% uuringusse kutsututest). *H. pylori* infektsiooni reinfektsioonikordaja oli 6,7% (95% CI 2,5–14,5%) inimaasta kohta. Kumulatiivne reinfektsioonimäär oli keskmiselt 6,6-aastase jälgimisperioodi lõpuks 37,5% (95% CI 15,2–64,%). *H. pylori* reinfektsiooni riskifaktoreid ei selgunud.

Samal rahvastikul põhinevad uurimustulemused *H. pylori* levimuse dünaamika kohta ja *H. pylori* ravijärgse reinfektsiooni riski kohta võimaldasid läbi viia arvutused, et võrrelda oletatavaid *H. pylori* infektsiooni levimuse muutusi laste hulgas: ühelt poolt neid, mis toimuksid rahvastikus laialdase *H. pylori*-vastase ravi rakendamise järgselt ja teiselt poolt neid, mis toimuksid aja jooksul

*H. pylori* nakatumisriski vähenemise tõttu. Kui sihtrühmas, kellele kavatsetakse rakendada *H. pylori* vastast antibakteriaalset ravi, oleks *H. pylori*-positiivseid 50%, ravi efektiivsus ja ravisoostumus oleksid mõlemad 80% ning reinfektsioonikordaja oleks 6% inimaastas, siis vahetult pärast ravi oleks selles sihtrühmas *H. pylori* infektsiooni levimusmäär 18% ning 11 aasta möödudes oleks see 34%. Teise stsenaariumi järgi toimuks vaid loomulik *H. pylori* infektsiooni levimuse vähenemine nakatumisriski vähenemise tõttu. Kuna 11 aasta jooksul on Eestis laste ja noorukite *H. pylori* seropositiivsuse šanss vähenenud 1,92 korda, siis järgmise 11 aasta möödudes oleks infektsiooni levimusmäär vastavas vanuserühmas langenud 50%-lt samuti 34%-ni. Võime järeldada, et *H. pylori* infektsiooni levimuse spontaanse vähenemise kiirus Eesti laste hulgas on võrreldav laialdase *H. pylori*-vastase ravi tulemusel saadava levimuse vähenemisega.

Kahekümnest lapsest, kes osalesid veise immuunkolostrumi efektiivsuse hindamise uuringus, käis 2 nädala jooksul pärast ravi lõppu kontrollil 16 last (80%). Mitte ühelgi patsiendil ei saavutatud immuunkolostrumi manustamisega *H. pylori* infektsiooni eradikatsiooni. Siiski oli täheldatav tendents kroonilise gastriidi paranemisele: kui enne ravi oli mao antrumi limaskestas 3. astme krooniline põletikuline infiltratsioon 8 lapsel, siis pärast ravi ei olnud nii tõsist põletikulist leidu enam mitte ühelgi lapsel ($p<0,05$).

## Järeldused

1. *H. pylori* infektsiooni levimusmäär Lõuna-Eestis elavate 9–15-aastaste laste hulgas oli 56%, mis on oluliselt kõrgem kui sama vanuserühma lastel Skandinaavias ning Lääne-Euroopa riikides. *H. pylori* infektsiooni levimus oli kõrgem maal elavate laste kui linnas elavate laste hulgas.
2. *H. pylori* infektsiooni levimus on laste hulgas Eestis pärast taasiseseisvumist 1991.a. aset leidnud suurte sotsiaal-majanduslike muutuste perioodil oluliselt vähenenud: vanusele standarditud *H. pylori* infektsiooni levimusmäär oli 1991.a. 42,2% ning 2002.a. 28,1% ($p<0,0002$). *H. pylori* levimusšanss on 11 aasta jooksul vähenenud 1,92 (95% CI 1,33–2,76) korda.
3. *H. pylori* reinfektsioonikordaja lastel ja noorukitel pärast kombineeritud antibakteriaalset ravi oli 6,7% inimaasta kohta, mis on suurem kui nende eakaaslastel Lääne-Euroopas ja Jaapanis. Kumulatiivne reinfektsioonimäär oli keskmiselt 6,6 aastat kestnud jälgimisperioodi lõpuks 37,5%. *H. pylori* reinfektsiooni riskifaktoreid ei selgunud.
4. Veise immuunkolostrumi manustamisega ei saavutatud *H. pylori* infektsiooni eradikatsiooni mitte ühelgi lapsel, siiski oli täheldatav tendents kroonilise gastriidi paranemisele.

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